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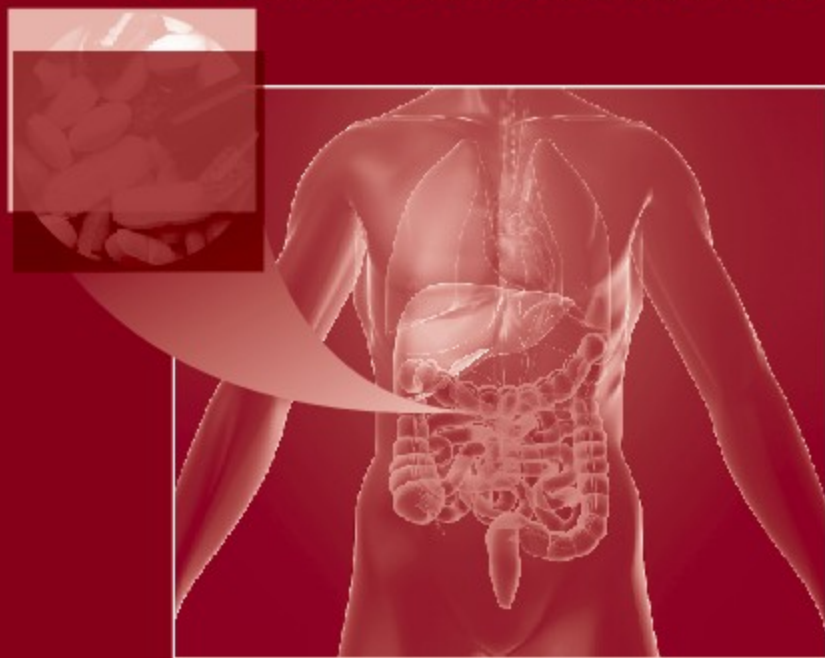
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SECOND EDITION



Oral Drug Absorption

Prediction and Assessment



Edited by

Jennifer B. Dressman
Christos Reppas

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Oral Drug Absorption

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Oral Drug Absorption

Prediction and Assessment

Second Edition

Edited by

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Informa Healthcare USA, Inc.
52 Vanderbilt Avenue
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Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number-10: 1-4200-7733-3 (Hardcover)
International Standard Book Number-13: 978-1-4200-7733-9 (Hardcover)

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Library of Congress Cataloging-in-Publication Data

Oral drug absorption : prediction and assessment / edited by Jennifer B. Dressman, Christos Reppas. — 2nd ed.

p. ; cm. — (Drugs and the pharmaceutical sciences ; 193)

Includes bibliographical references and index.

ISBN-13: 978-1-4200-7733-9 (hardcover : alk. paper)

ISBN-10: 1-4200-7733-3 (hardcover : alk. paper) 1. Oral medication. 2.

Bioavailability. I. Dressman, J. B. (Jennifer B.) II. Reppas, C. (Christos) III.

Series: Drugs and the pharmaceutical sciences ; 193.

[DNLM: 1. Pharmaceutical Preparations—metabolism. 2. Administration, Oral.

3. Biological Availability. 4. Solubility. W1 DR893B v.193 2010 / QV 38 O63 2010]

RM162.0727 2010

615'.6—dc22

2009052260

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“To Torsten and Vicky, the wings beneath our feet”

Preface

Ten years ago, the first edition of *Oral Drug Absorption* was published with the intent of closing the gap between gastroenterology textbooks on the physiology of the gastrointestinal (GI) tract and pharmaceutical textbooks on oral drug formulations. In the ensuing years, the field of oral drug absorption has evolved significantly on several fronts. First, there has been acceptance and increasing implementation of the biopharmaceutics classification scheme (BCS) concept, both at the regulatory and drug development levels. Second, application of biorelevant media to better understand active pharmaceutical ingredient (API) behavior in the GI tract has become widespread, not only for solubility and dissolution, but also for permeability applications. Third, the role of transporters in drug uptake across the GI mucosa has been recognized and is under intensive investigation. The same applies to drug metabolism in the gut wall, and within the next decade, we should be able to quantitatively describe both of these phenomena as they relate to prediction of API bioavailability. Fourth, our understanding of GI hydrodynamics and the availability of fluid in the various segments of the GI tract is slowly but surely improving. Fifth, there is a very strong interest in applying physiologically based pharmacokinetic (PBPK) modeling to oral drug absorption; this has become possible with the advent of sophisticated software programs like GastroPlus[®] and PK-Sim[®] and will surely become one of our most powerful tools in the years to come. And last but not least, the quality by design paradigm has aroused interest in new techniques to better link the composition and manufacture of oral drug products with their in vivo performance.

As a result of all these developments, it is high time to bring out a second edition of *Oral Drug Absorption* that captures the rapid progress in the field. In this edition, we start out with a chapter on the fundamentals of GI physiology—as it relates to oral drug absorption—and in the first section, we describe several aspects in more detail in chapters specifically addressing absorption mechanisms, GI motility, gut wall metabolism, food effects, and drug absorption in children and various disease states. The second section focuses on the BCS and the impact it is making on pharmaceutical R&D and regulation of oral drug products. Additionally, separate chapters are devoted to the measurement and interpretation of the key BCS parameters, solubility and permeability. The third section, entitled “Nonclinical Methods to Evaluate Oral Formulations,” first describes appropriate dissolution tests to characterize formulations in pre-clinical development—be they intended for immediate or controlled release in the GI tract. Then state of the art practice for formulation screening and development in the industry is recounted for immediate release products and for controlled release products. This section is wrapped up by a chapter devoted to implementation of PBPK modeling at the preclinical level. The last section turns attention to bioequivalence studies. Increasingly, alternatives to human pharmacokinetic studies are being used to obtain approval of generic drug

products and to obtain continued approval for existing products when changes have been made to the composition or method of manufacture. The section is introduced with a chapter providing the fundamentals and current status of bioavailability and bioequivalence. The application of BCS and in vitro–in vivo correlation (IVIVC) to the proof of bioequivalence is then described in separate chapters, and the evolution of regulations pertaining to bioequivalence is put into global perspective. In recognition of the increased interest in application of IVIVC for proof of bioequivalence, we have also provided a CD, which shows the user how to generate IVIVCs utilizing an Excel spreadsheet. Numerous examples are given to illustrate the underlying theory and show how the IVIVC works, and it is hoped that this CD will become an integral part of the toolbox used by pharmaceutical scientists to facilitate formulation design and optimization on a day-to-day basis.

Of course, producing a new edition of a textbook requires the assistance of many people, and *Oral Drug Absorption* is no exception. We wish to thank Sandy Beberman and Sherri Niziolek for their untiring enthusiasm and support of this project. We are also indebted to the authors for their splendid efforts in preparing chapters that reflect state-of-the-art thinking in oral drug absorption. We also wish to thank our families for their support and understanding that books are largely created in the evenings and on weekends. Finally, we thank the readers of the first edition for the excellent feedback and stimulus to produce an updated version.

Jennifer B. Dressman
Christos Reppas



Contents

Preface ix

Contributors xiii

Part I: Physiology of Oral Drug Absorption

1. Physiological Factors Affecting Drug Release and Absorption in the Gastrointestinal Tract 1

Erik Söderlind and Jennifer B. Dressman

2. Drug Transport Mechanisms Across the Intestinal Epithelium 21

Anna-Lena B. Ungell

3. Gastrointestinal Transit and Drug Absorption 41

Clive G. Wilson, Werner Weitschies, and James Butler

4. Gut Wall Metabolism 66

Mary F. Paine

5. Food Effects on Drug Absorption and Dosage Form Performance 90

Anette Müllertz

6. Oral Drug Absorption in Pediatric Populations 108

Andrea N. Edginton and Nikoletta Fotaki

7. Gastrointestinal Disease and Dosage Form Performance 127

Vladan Milovic and Jürgen Stein

Part II: The Biopharmaceutics Classification System

8. The Biopharmaceutics Classification System: Recent Applications in Pharmaceutical Discovery, Development, and Regulation 138

Jennifer J. Sheng and Gordon L. Amidon

9. Drug Solubility in the Gastrointestinal Tract 155

Christos Reppas and Patrick Augustijns

10. Permeability Measurement 168

Joachim Brouwers, Sven Deferme, Pieter Annaert, and Patrick Augustijns

11. BCS: Today and Tomorrow 206

James E. Polli

Part III: Nonclinical Methods to Evaluate Oral Formulations

- 12. Dissolution Testing to Forecast In Vivo Performance of Immediate-Release Formulations** 224
Ekarat Jantratid and Maria Vertzoni
- 13. Dissolution Testing to Forecast the In Vivo Performance of MR Formulations** 244
Sandra Klein
- 14. Modified-Release Dosage Forms: Formulation Screening in the Pharmaceutical Industry** 265
Bertil Abrahamsson and Erik Söderlind
- 15. Immediate Release Oral Dosage Forms: Formulation Screening in the Pharmaceutical Industry** 296
Yunhui Wu and Filippas Kesisoglou
- 16. Computer Models for Predicting Drug Absorption** 338
Neil Parrott and Thierry Lave

Part IV: Bioequivalence Studies

- 17. In Vivo Bioequivalence Assessment** 356
Panos Macheras and Mira Symillides
- 18. Biowaiving Based on the BCS—A Global Comparison** 372
Henrike Potthast
- 19. Biowaiving Based on In Vitro-In Vivo Correlation** 386
Vinod P. Shah

Appendix 395
Frieder Langenbucher

Index 397



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Physiological Factors Affecting Drug Release and Absorption in the Gastrointestinal Tract

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INTRODUCTION

In vivo drug release from oral drug formulations may be affected by a number of physiological factors including volume and composition of gastrointestinal (GI) fluids, the pH and buffer capacity of these fluids, digestive enzymes, contraction patterns, and bacterial flora in the gut. In addition, the extent of drug absorption and bioavailability may be further affected by GI transit, the presence of cellular transporters, and metabolic enzymes.

Several of those factors are affected by intake of food. The effects of food on the physiology, and consequently the in vivo drug release and absorption, are most pronounced in the stomach. In fact, even coadministration of water with a dosage form may influence the conditions in the stomach as a result of dilution effects. The food effects become less significant further down the GI tract but should not be disregarded.

In this chapter, the physiological factors most relevant to drug release and absorption are described and the effects of food intake concomitant with administration of dosage forms are discussed.

OVERVIEW OF THE GI TRACT

Functions of the GI Tract

The GI tract serves as the portal for supplying solid and liquid nutrients to the body. Food and drinks are processed by the digestive tract into more absorbable forms and are also brought to the main absorptive sites at a measured rate, so as to not overload the gut's capacity to absorb them. Materials that have not been digested by the time they reach the lower end of the small intestine may be subjected to fermentation by the bacteria that reside in the lower bowel. If not, they are excreted in the stool, along with cells sloughed off from the mucosal lining of the GI tract, dead bacterial cells, and other waste materials.

In addition to its digestive and absorptive functions, the GI tract also plays an important role in homeostasis. It has been calculated that about 9 L of fluid enter the GI tract each day, of which only about 2 L are ingested orally. The remaining fluids are secreted from various segments in the GI tract as well as from organs that supply the GI tract with substances that are crucial to digestion, for example, the gall bladder and pancreas. The fluids are largely reabsorbed in the lower jejunum and ileum (about 80–90%) and, apart from the stool water (about 200 mL), the rest is reabsorbed in the colon. Disruption of this

reabsorption function, for example, by pathogens and their toxins, results in diarrhea.

Although the main function of the GI tract is to facilitate absorption of nutrients necessary for metabolism and energy, it must also prevent absorption of unwanted materials, such as viruses, bacteria, and noxious substances. A relevant factor in this respect is that the digestive processes are able to attack and degrade most foreign proteins, thus minimizing the possibility that they will be absorbed intact. Another relevant factor is that, unlike in many other organs in the body, the absorptive mechanisms in the GI tract are hostile to the uptake of macromolecules. Further, the gut has its own local immune system. Sampling of foreign bodies through contact with Peyer's patches can lead to production of IgA antibodies and rapid neutralization of the perceived pathogen by the next contact.

Last but not the least, the GI tract plays a role in the elimination of some compounds. For example, hepatic metabolites may be eliminated into the bile and pass into the GI tract when the gall bladder contracts. Of course, some compounds secreted in this way may be (partly) reabsorbed through the gut wall, a process often referred to as enterohepatic cycling, but some will pass on through the gut into the feces. Other compounds may be directly eliminated through the gut wall via the efflux pump, P-glycoprotein (1); classical examples being verapamil and digoxin.

It is in the context of this background that we, as pharmaceutical scientists, attempt to deliver drugs. In advantageous cases, the drug will be released completely from the dosage form, escape decomposition by stomach acid and the digestive and fermentative processes, be a substrate for the uptake processes available at the site(s) where it is released, and be efficiently absorbed into the systemic circulation. In disadvantageous cases, the drug will not be completely dissolved in the gut, may be subject to decomposition, and only poorly permeate the gut wall. Through clever formulation we strive to take even poor candidates for oral absorption and turn them into efficiently absorbed drugs. Knowing where a new chemical entity stands in the spectrum of good versus poor candidates is, of course, a prerequisite to formulation development and requires not only a good understanding of the physical chemistry of the drug substance but also the environment in which it is to be delivered—the GI tract.

Dimensions of the GI Tract

Figure 1 shows a schematic of the GI tract, divided into its most important segments: the stomach, the small and large intestine, and the organs that supply it with secretions (the liver via the gall bladder, and the pancreas).

The volume of the stomach adjusts to meal intake. While the resting volume of gastric fluids is only around 30 to 50 mL (corresponding essentially to a moist mucosal surface), the stomach can expand without difficulty to accommodate up to 1 to 1.5 L of food, ingested fluids, and secretions after a meal. Immediately upon ingestion of the meal, the volume of the gastric contents represents the volume of contents ingested. After an initial emptying of some of the meal fluid there is a period in which the volume of gastric contents remains essentially constant, during which gastric secretions balance out gastric emptying of the chyme. Later in the postprandial phase (about 2–3 hours after meal intake), gastric emptying becomes predominant and the volume of gastric contents starts to decrease again (2).

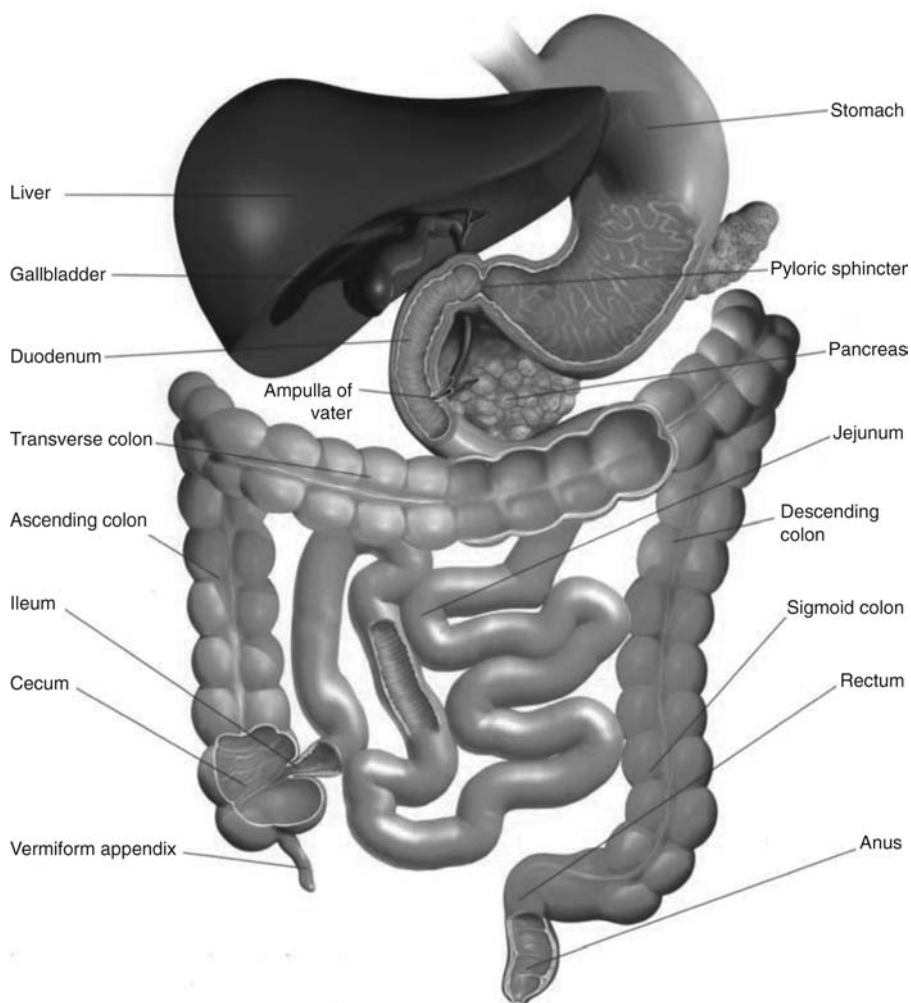


FIGURE 1 Anatomy of the gastrointestinal tract. *Source:* Courtesy of Capsugel, a division of Pfizer, Inc.

The small intestine is typically 3 to 5 m long in adults and has a diameter of about 3 to 4 cm at the proximal end, tapering to about 2 to 3 cm at the distal end. It can be conveniently divided into the duodenum, jejunum, and ileum for descriptive purposes. The duodenum is essentially a mixing segment, bringing together the incoming chyme from the stomach and secretions from the gall bladder and pancreas at the level of the ampulla of Vater. The ligament of Treitz represents the transition from the duodenum to the jejunum. The jejunum is about 100 to 150 cm long and digestion and absorption of nutrients occur to the greatest extent here. The ileum makes up the rest of the small intestine and in this region there are specific mechanisms for the reuptake of bile salts and a few

nutrients, most notably vitamin B12. Additionally, Peyer's patches can be found in this region and on into the colon.

Although the proximal small intestine is essentially sterile in healthy individuals, microbial numbers climb with approach to the ileocecal valve, reaching numbers as high as 10^3 to 10^4 colony-forming units per milliliter by the end of the ileum. The cecum and appendix play only a very minor role in human GI physiology compared to other species and are widely considered to be vestigial.

As reflected in the name, the diameter of the large intestine is considerably greater than that of the small intestine—up to 6 to 8 cm. The colon (large intestine) is altogether about 1.5 m long and, analogous to the small intestine, can be divided into three principal regions: the ascending (proximal), transverse, and descending colon. Of these three, the most interesting for dosage form design is the ascending colon, since in addition to the higher volume of fluids available, this segment has a comparatively reliable residence time. In the transverse colon the stools start to form and passage times through this and the descending colon are extremely variable, ranging from a few minutes to many hours.

Overview of GI Transit Times

After being swallowed, the dosage form moves through the esophagus into the stomach. In young, healthy individuals the passage time through the esophagus is short, on the order of seconds, as long as the dosage form is ingested with adequate fluid in a standing or sitting position. For supine patients, or when just a few milliliter of fluid is ingested with the dosage form, it may take several minutes for the dosage form to pass through the esophagus and drug release may occur in this region resulting, in the worst case, in excoriation of the delicate esophageal mucosa and possible ulceration. In elderly individuals, the swallowing process often becomes less coordinated, resulting in reduced ability to clear the dosage form with the swallow and in this subpopulation the risk of premature drug release in the esophagus is commensurately higher.

The gastric passage time is highly variable, with emptying of gastric contents dependent on a number of factors, ranging from physicochemical parameters such as pH, temperature, calorie content, volume, and viscosity of the contents to physiological influences such as the phase of the migrating motility complex in the fasted state and its conversion to a distinctly different motility pattern when a meal is ingested. Values for emptying of dosage forms from the stomach can easily range from just a few minutes for a warm, isotonic, noncalorific fluid in the fasted state to many hours for a nondisintegrating tablet swallowed after consuming a calorie and fat-rich meal.

By contrast, the passage time through the small intestine is much less variable, ranging from about three to five hours in healthy adults irrespective of meal intake and dosage form format. In the small intestine, motility is partly segmenting, partly propagative in nature, with the net result that the contents move aborally (i.e., from proximal to distal) over time.

In the ascending colon, movement of the contents in both directions is common, resulting in the possibility that items that have been more recently ingested may actually be found lower in the colon than items ingested before them (3). Estimates of passage times through the proximal colon range from about 5 to 12 hours. As mentioned earlier in this chapter, passage times through

the remaining colonic segments can be erratic. Stimulation of the gastrocolic reflex by meal ingestion may result in a bolus movement from the start of the transverse colon to the rectum within a few minutes. On the other hand, defecation frequencies of only two or three times a week are still considered to be within the normal range, implying that passage time through the lower colon can easily exceed 48 hours in healthy adults. The reader is referred to chapter 3 (by Wilson et al.) for a more in-depth discussion of passage times in the GI tract and their implications for oral drug delivery.

STOMACH

Role and General Description of the Stomach

The stomach performs several functions that are important to assimilation of foodstuffs and to defence mechanisms in the GI tract. Since humans, unlike herbivores, usually ingest food in discrete meals rather than grazing, the stomach plays an important role in regulating the rate of transfer of foodstuffs into the small intestine, thus preventing an overload of the digestive capacity in that region. To fulfill this reservoir function, the folds of the stomach (*rugae*) can relax to accommodate meals as large as 1 to 1.5 L without causing discomfort. The meal is mixed with the gastric secretions in the body and antrum of the stomach and the particle size of the meal solids is reduced, so that solid meal residues are reduced to a particle size predominantly less than 1 mm before being emptied from the stomach (4). Meal emptying appears to be primarily regulated by the caloric content, with a typical rate of emptying about 2 to 4 Kcal/min in a healthy adult. Other factors such as pH, viscosity, temperature, and fat content of the meal can modulate this rate somewhat. So typical meals containing several hundred Kilocalories will take several hours to empty from the stomach and will do so in a relatively zero-order fashion (with a lag time to reduce the solid particle size if the meal has not been well masticated).

Some digestion takes place during the residence time in the stomach: pepsin initiates protein digestion while gastric lipase accounts for 15% to 20% of total fat digestion. Gastric acid aids and abets digestion of proteins through denaturation and also plays a role in the host defense mechanisms by inactivating many types of bacteria. It is also required for the activation of pepsin from pepsinogen (the precursor form in which pepsin is secreted).

After the bulk of the meal has been emptied from the stomach and only a few residues remain, which cannot be further reduced in particle size by the digestive and contractile functions of the fed stomach, the fasted state motility pattern resumes (see sect. "Stomach—Motility and Transit"). This fasted motility pattern includes a brief spurt of very intensive contractions about once every two hours, the so-called housekeeper wave, which clears any remaining residues out of the stomach. With the advent of a housekeeper wave, non-disintegrating dosage forms are also emptied from the stomach into the small intestine.

Cell Types and Functions

The mucosa of the stomach and its associated crypts (Figs. 2–5) contain a variety of cell types with a wide array of functions. The surface mucosa consists of squamous/columnar epithelial cells that produce and secrete bicarbonate ion

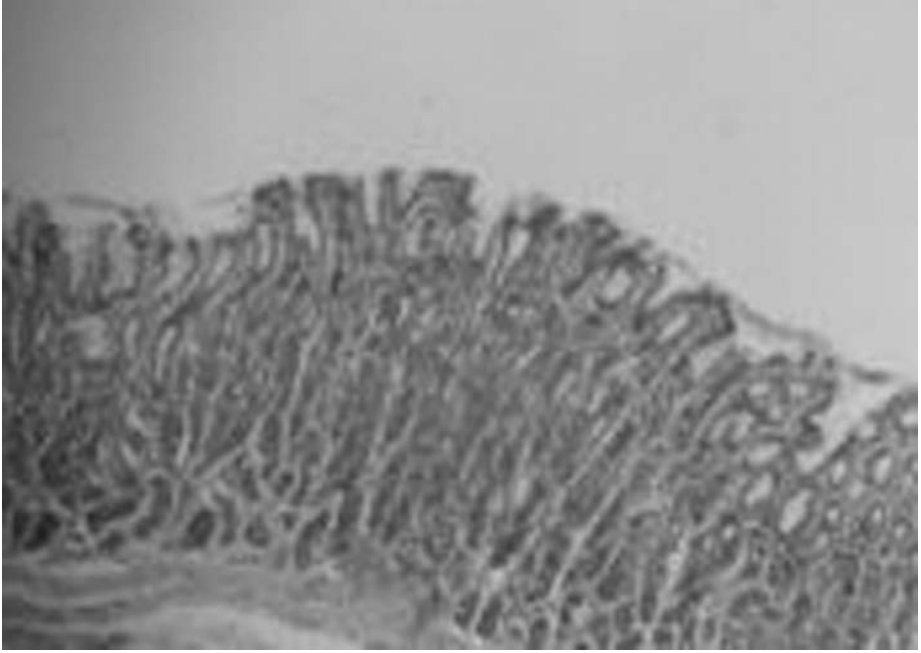


FIGURE 2 Gastric mucosa in cross section.

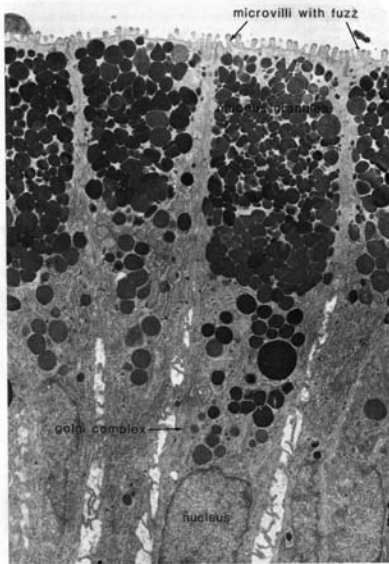


FIGURE 3 Gastric mucus cells. Reproduced with permission from Johnson LR. *Physiology of the Gastrointestinal Tract*. 2nd Ed. Raven Press, p824.

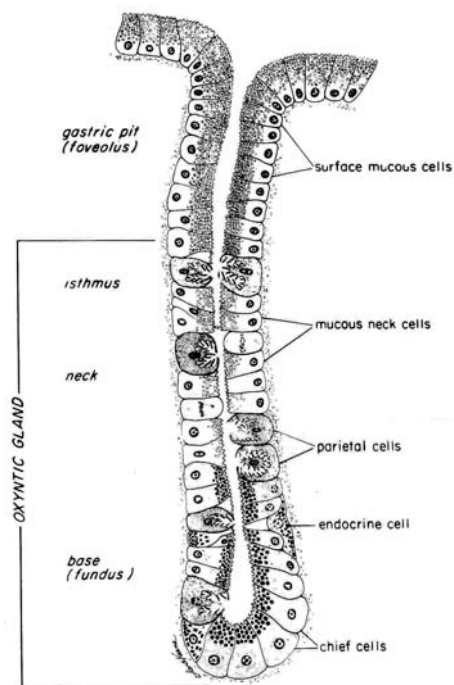


FIGURE 4 Cross section of an oxyntic gland showing the position of the parietal and Chief cells. *Reproduced with permission from L.R. Johnson, Physiology of the Gastrointestinal Tract. 2nd Edition, Raven Press, p819.*

and mucus on the apical side. These secretions form a mucus layer that is buffered to a neutral pH at the cell surface and thus protects the cells against excoriation by gastric acid and pepsin as well as the potentially harmful substances we ingest (alcohol, highly spicy foods, etc.). It should be noted that this surface mucosa does not possess the key features important to efficient drug absorption that are found in the small intestine: there are no villi and the microvilli on the surface mucosal cells are few and underdeveloped. Thus, the effective surface area for absorption can essentially be calculated from the geometry of the stomach. Together with the lack of transporters in this region and variable transit times, the relatively low surface area results in the stomach being an unreliable and inefficient site of drug absorption.

Gastric acid is secreted by parietal cells, which are located in the crypts of the gastric mucosa. In these cells, which are located primarily in the fundus and corpus of the stomach, hydrochloric acid is produced and stored in intracellular vesicles (Fig. 4). These vesicles can be rapidly transformed into secreting channels upon stimulation by gastrin, a hormone that is released in response to the meal, resulting in an almost immediate and quite powerful acid output (up to 25 mM/hr) compared to the basal rate of secretion that is about 1.5 mEq/hr in women and 2.5 mEq/hr in men. In addition to gastric acid, parietal cells also produce intrinsic factor, which is necessary for the assimilation of vitamin B12.

A third type of cell in the gastric mucosa is the Chief cell, which produces lipase and the precursor enzyme, pepsinogen. Like parietal cells, Chief cells are found in the crypts of the gastric mucosa and, when stimulated by meal intake, secrete the (pro)enzymes in much greater concentrations into the lumen of the stomach.

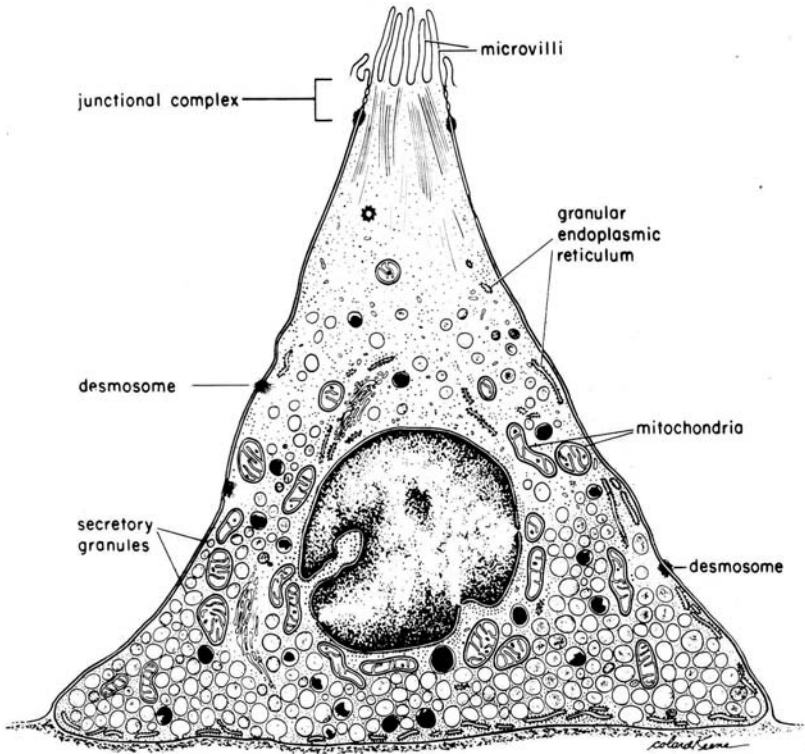


FIGURE 5 G cells are located in the antral mucosa. Reproduced with permission from L.R. Johnson, *Physiology of the Gastrointestinal Tract*. 2nd Edition, Raven Press, p844.

The fourth important cell type is the G cell, which produces the hormone gastrin. Like the Chief and parietal cells, the G cells are located in the crypts. In contrast to the Chief and parietal cells, the G cells are found primarily in the antrum of the stomach and gastrin is transported via the local circulation to the parietal cells rather than secreted into the gastric lumen (Fig. 5).

Composition of Gastric Fluid

The most distinctive property of the fluids in the fasted state stomach is their low pH. Determinations of pH in gastric aspirates or by radiotelemetry methods have shown that the fasted state pH is typically below 2 but can range between 1 and 7.5 (5–10). The buffer capacity of the fasted state gastric fluid has been determined only rarely and reported median values range between 7 and 18 mmol/L $\times \Delta\text{pH}$ (10).

Intake of food results in an almost instantaneous increase of the gastric pH. Depending on the contents of the meal, the fed state gastric pH increases to values between 4 and 7 (2,5,10,11). Soon after food intake the gastric pH starts returning gradually to the fasted state pH. After a solid meal the fasted state pH is reached in approximately two hours. Interestingly, after a liquid meal, that is, a nutritional drink, the gastric pH appears to remain elevated longer (typically >4 hours) than after a solid meal. The buffer capacity of the fed state gastric fluid

is reported to be higher than in the fasted state, but it is to a large extent determined by the contents of the meal (10).

The dominant ion in the fasted state gastric fluid is chloride, followed by sodium and potassium. Reported mean concentrations of these ions are 102 mM (chloride), 68 mM (sodium), and 13 mM (potassium), with a corresponding mean ionic strength of 0.1 M (7). Another important component in the gastric fluid is pepsin, a digestive enzyme that is released in the stomach and that hydrolyzes proteins. Reported mean values of the pepsin concentration range from 0.1 to 1.3 mg/mL, depending on whether water was administered prior to sampling of gastric fluid (10). The pepsin concentrations seem to be slightly higher in the fed state (0.3–1.7 mg/mL) (10).

All dissolved materials contribute to the gastric fluid osmolality, but the content is nevertheless usually hypoosmotic in the fasted stomach. The osmolality in the fasted state depends greatly on the amount of water given prior to investigation, and ranges from 30 to 280 mOsm/kg (7,10). Osmolality values of greater than 100 mOsm/kg appear to be the most common.

It should be noted that the surface tension of fasted state gastric fluid is clearly lower than that of aqueous solutions of simple electrolytes. Typically, surface tensions below 50 mN/m have been observed (9,10), whereas the surface tension of pure water is 72 mN/m. The surface tension of fed state gastric fluid may be even lower depending on the composition of the meal. The surface tension results clearly indicate the presence of surface active components in the gastric fluids, although these are yet to be conclusively identified.

Stomach—Motility and Transit

In the fasted state, the motility pattern in the stomach is regulated by the interdigestive migrating myoelectric complex (IMMC), which follows a three-phase cyclic pattern (12). These three phases have been designated phase I, a period of quiescence and essentially no movement of the gastric fluid, lasting about 45 to 60 minutes; phase II, consisting of 30 to 45 minutes of irregular activity that favors dissolution in the stomach; followed by phase III, a period of 2 to 10 minutes of intense contractile activity during which the entire stomach content is emptied into the small intestine (the “housekeeper wave” referred to in sect. “Overview of GI Transit Times”). The motility cycle is initiated in the stomach, typically in the corpus region, and passes progressively along the small intestine into the distal ileum. As one cycle is terminating in the distal ileum, the next is already beginning in the stomach.

Ingestion of food interrupts the interdigestive cycle and the motility pattern becomes more regular. Depending on caloric load and specific nutrient content of the meal, this period of mild to moderate contractions may last several hours. The presence of food not only modifies the motility pattern of the stomach but the viscosity of the gastric fluid is also likely to increase. Consequently, the shear forces on solid dosage forms may increase, possibly resulting in higher dissolution/release rates, particularly for formulations where dissolution is erosion-controlled (13).

The gastric residence time of a solid dosage form depends on the size of the dosage form and whether or not the formulation is taken with a meal (14,15). In the fasted state, small solids (<2 mm) may empty from the stomach during all IMMC phases, with mean gastric half-lives typically less than one hour. For

larger solids (>2 mm) the gastric emptying is dependent on the phase of the motility cycle, requiring the advent of a phase III burst to be emptied from the stomach. Depending on the timing of dosage form ingestion vis à vis the next housekeeper wave, gastric residence times of more than one hour are possible for larger, nondisintegrating dosage forms.

In the presence of food, small solid particles empty more slowly than in the fasted state. The gastric residence time increases and becomes more variable. The mean gastric half-life may increase to considerably longer than two hours, depending on the composition of the meal. The effect of food on the residence time of larger solids is more pronounced. After a very heavy meal, nondisintegrating tablets have been retained in the stomach for over 14 hours (13).

SMALL INTESTINE

Role and General Description of the Small Intestine

The small intestine is the main site of digestion and assimilation of nutrients into the body within the GI tract. As chyme is passed out through the pylorus into the duodenum, it is mixed with the bile and pancreatic juice, both of which facilitate the digestive process. The pancreatic juice contains enzymes that can digest carbohydrates (amylase), proteins (proteases), and fats (lipases), as well as bicarbonate ion that serves to neutralize the incoming acid from the stomach and thus provides more optimal pH conditions for the pancreatic enzymes.

Most starches can be digested by amylase into disaccharides, which are then cleaved at the mucosa and transported into the enterocytes as monosaccharides by an active process, leading to rapid and complete assimilation of sugars and starches in the upper small intestine. However, some types of polysaccharide fibers, for example, celluloses, are not digested by amylase and continue through the small intestine intact. Protein digestion starts in the stomach and is very efficient in the upper small intestine, so protein assimilation is usually completed within the first 100 cm of the small bowel. There are a variety of transporters for the active uptake of amino acids, dipeptides, and tripeptides into the enterocytes, but not for larger peptides. Fats and oils, already partly emulsified and digested in the stomach, are further digested by the pancreatic lipases. The role of the bile is to provide a large interfacial contact area between the lipases and their substrates, thus improving the efficiency of fat digestion. The products of fat digestion, free fatty acids and monoglycerides, can be transported into the enterocytes by passive mechanisms, whereupon they can be packaged into chylomicrons in the cell interior and then typically transported into the general circulation via the lymph.

The architecture of the small intestine, as shown in Figure 6, is ideal for absorption of nutrients, providing a huge area of surface contact between the nutrients and the absorbing mucosa. First, the folds of Kerckring provide about a threefold increase in the surface area vis à vis the corresponding geometrically derived surface area. Second, the defining feature of the small intestine, the villi, provides another increase of about eight- to tenfold in surface area, and third, the microvilli on the apical side of the enterocytes further expand the surface area by a factor of up to 20. Several authors have suggested that the effective absorptive surface area of the small intestine in a healthy adult might be as high as 200 to 500 m² (16,17), comparable with that of a tennis court. Such a large surface area is, of course, extremely conducive to absorption. For example, the

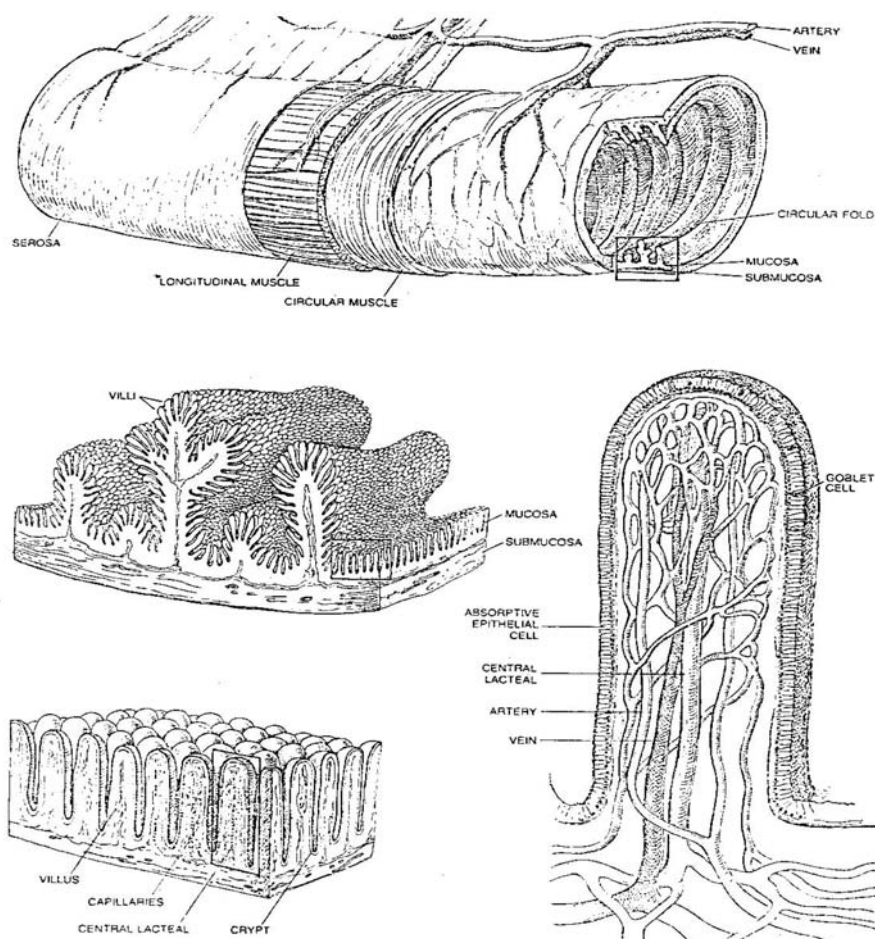


FIGURE 6 Architecture of the small intestine. *Source:* From Ref. 17.

rate of uptake via diffusion is directly proportional to the surface area across which the diffusion occurs. Furthermore, the nutrients have to travel across only a one-cell thick layer to access the fine capillary network of the intestinal circulation or to come in proximity to the lymphatic lacteals that extend into the middle of the villi.

Depending on the composition of the meal and fluid intake with the meal, the chyme can be hypotonic or hypertonic. Should the chyme be hypertonic, a net flux of water from the mucosa into the lumen of the small intestine will occur in an attempt to reestablish isoosmotic conditions. Conversely, if the chyme is hypotonic, water flux across the mucosa will occur from the lumen into the mucosa. In addition to these effects, water transport will also occur secondary to nutrient uptake, again resulting in water flux from the lumen into the mucosa. Net water transport results from a combination of these and other influences. Looking at the small intestine as a whole, about 9 L of fluid enters the small intestine in the course of a day, about 7 L of which is reabsorbed by the ileocecal

junction. So the small intestine can be regarded as a “large capacity, low efficiency” (at least relative to the colon) site of water reuptake, and therefore plays a key role in water homeostasis in the body. Apart from water reuptake, bile salts are also reabsorbed in the small intestine, passively at the level of the jejunum and actively in the ileum.

Absorptive Mechanisms in the Small Intestine

Each villi is covered with a single layer of cells, consisting of about 80% to 90% specialized absorptive cells and 10% to 20% goblet cells, as well as minor number of endocrine and exocrine cells in the crypts associated with the villus. Mucous production by the goblet cells helps protect the delicate mucosa from injury by the digestive enzymes and any harmful substances ingested with the meal. The absorptive cells are columnar in appearance with a depth of about 35 μm . They are produced as undifferentiated cells in the crypts and migrate with time up and along the villus, with an average crypt-to-tip migration time of about 48 hours. By about two-thirds the way up the villus they reach full metabolic maturity, and once they reach the tip of the villus they are sloughed off into the lumen and digested/excreted with the stools. The absorptive cells have a number of features that facilitate uptake of digestive products and drugs. First, microvilli are present on the apical side, contributing to the surface area advantage in this segment of the GI tract. Second, the apical membrane is a lipophilic double-layer membrane and thus conducive to partitioning of lipophilic materials (which nowadays corresponds to the physicochemical properties of many drug substances). Third, embedded in this membrane are a number of carrier molecules that, upon recognition of a substrate, can facilitate transport of the drug substance into the enterocyte. In some cases corresponding carriers may also be present on the basolateral membrane. Yet another pathway for absorption across the small intestinal mucosa is the so-called paracellular pathway, that is, between the enterocytes instead of through them. Although quite narrow and therefore very restrictive in terms of molecular size cutoff, this paracellular pathway offers an opportunity for highly hydrophilic molecules to pass through the small intestinal mucosa.

It should be noted that there are gradients within the small intestinal mucosa for all of these mechanisms of uptake. Many of the carriers are more prevalent in the proximal part of the small intestine (e.g., amino acid, and peptide transporters), while others are more prevalent in the distal part (e.g., those for bile salts). There is also a surface area gradient, since the folds and villi are more pronounced proximally than distally and the diameter of the small intestine itself tapers with distance from the pylorus. So passive, diffusional uptake through the enterocytes (transcellular passive absorption) occurs more efficiently in the proximal part of the small intestine. The paracellular pathway “pores” (often referred to as “tight junctions” between the cells) are wider at more proximal locations, affording access to molecules up to about 300 Da, and become more restrictive with distance from the pylorus. In fact, in the large bowel, the tight junctions are so restrictive that only very small molecules such as urea can pass through by this mechanism. Some drugs can be taken up by both paracellular and transcellular mechanisms. If this is the case, typically the transcellular uptake will be the more important mechanism, since less than 1% of the entire surface area of the mucosa consists of the tight junctions.

It should also be noted that not all carriers facilitate uptake into the enterocytes. As part of the host defense system, there are also carriers, for example, the P-glycoproteins, that discharge molecules from the enterocytes back into the GI lumen. This is, of course, contraproductive to the overall absorption of the drug. In addition to push back by these exotransporters, many compounds can be metabolized in the enterocytes. The metabolism at the interface between the GI lumen (essentially still “external” to the body) and the body interior also helps to protect the body from noxious materials. This topic is covered in much more detail in chapter 4 (by Paine).

Once the drug has passed through the monolayer on the villus, it can almost immediately pass through fenestrations into the fine capillary network that exists in each villus. From there the capillaries feed into the veins, which are collected into the portal vein. This means that most absorbed substances will pass through the liver on the way to the general circulation. The exceptions are very highly lipophilic molecules ($\log P$ of about 6 and higher), which may participate in chylomicron packaging. The chylomicrons are too big to squeeze through fenestrations into the capillaries and must instead diffuse further to the central lacteal, from where they are transported via the lymph, and are thus able to enter the general circulation at the left subclavian vein, that is, without having to pass through the liver. Lymphatic transport appears to be highly dependent on coingestion of fats—basal lymphatic flow is very low, but rates increase after meal intake, particularly if the meal is fatty.

In addition to the villi, there are also flat regions in the ileum and in the proximal colon, the so-called Peyer’s patches. It is at these regions that foreign particles can be sampled by the local immune system, after which they are either neutralized or stimulate production of antibodies. Although Peyer’s patch sampling contributes significantly to the local host defence system, there is very little evidence that a quantitative absorption of drugs can occur by this mechanism.

Small Intestine—Intestinal Fluid

The pH in the intestinal fluids has been determined by collecting aspirates or by radiotelemetry methods, the two methods giving similar results (5,7,10,18,19). The major determinants of the luminal pH in the small intestine are the pH of the gastric contents entering the small intestine and the buffering pancreatic secretion. Additionally, bicarbonate secretion along the small intestine results in a further rise in pH as the contents proceed toward the ileum.

In the fasting state, the pH in the proximal small intestine is highly variable and is largely determined by the interplay with the IMMC (20). During phase I (absence of motor activity), the pH remains stable at approximately 7. Phase II (irregular motor activity) is accompanied by a lowering of pH to values fluctuating between 2 and 7.5. During late phase II, the pH stabilizes at approximately 7 just prior to the phase III contraction and stomach emptying. However, the phase III activity appears to be delayed if the duodenal pH is too low (<4), and phase III activity will not commence until the duodenal pH exceeds 7. During phase III a neutral duodenal pH is maintained. As a result of the IMMC activity, determinations of the fasting state pH in the proximal small intestine often result in mean values of approximately 6.5, but can momentarily fluctuate between 2 and 7 (5,10). During meal digestion the three-phase cycle of the IMMC is interrupted and

the motor activity of the stomach becomes more regular. Prior to emptying of the stomach content into the small intestine, the duodenal pH is in the fasted state range, that is, at approximately 6.5. Postprandially, the pH in this region gradually decreases as increasingly acidic gastric content enters the intestine, before returning to fasting state values at the completion of the digestive period.

In the distal small intestine, ileum, the pH rises to approximately 7.5 as a result of bicarbonate secretion (18,19). Furthermore, the effects of food intake on pH diminish toward the distal end of the small intestine.

The buffer capacity of fasting state intestinal fluid ranges between 2 and 6 mmol/L $\times\Delta$ pH, which is somewhat lower than for gastric fluid (10,18,21). The buffer capacity of fed state intestinal fluid ranges between 13 and 30 mmol/L $\times\Delta$ pH, clearly higher than in the fasted state and most likely because of the buffering effect of food components and digestion products.

Various colloidal lipid phases are formed in the intestinal fluid by the combined action of the lipolytic enzymes secreted by pancreas and the components of the secreted bile. The intestinal fluid contains a mixture of mixed micelles, liposomes, and emulsion droplets formed by bile acids, cholesterol, and other lipids including glycerides, phospholipids, and free fatty acids. These colloidal aggregates have the ability to solubilize drugs, in particular lipophilic drugs, but the aggregates or their constituents may also interact directly with the dosage form or the formulation excipients. Both fed and fasted state intestinal fluids have been characterized with respect to their levels of bile acids and various lipids (10,18,21,22). In general, fasted state intestinal fluid contains 2 to 6 mM bile acids and low concentrations of neutral lipids (including 0.1 mM fatty acids) and phospholipids (0.2 mM). In the fed state, both the concentrations of the lipid colloid components and the variability of these values are higher. The reported concentration ranges are 4 to 37 mM for bile acids, 22 to 58 mM for neutral lipids (primarily fatty acids, mono- and diglycerides), and 3 to 6 mM for phospholipids. Due to extensive lipolysis of dietary lipids, the concentrations of mono- and diglycerides may be quite high, typically 2 to 7 mM.

As a consequence of the presence of surface-active components in the intestinal fluid, the surface tension is lowered to approximately 30 mN/m (10,18,21), both in fasting and fed state, which is expected to have a positive impact on the wetting of solid dosage forms.

The reported mean concentration of sodium ions in the intestinal fluid is 142 mM, making it the dominant ion in the small intestine. The reported mean concentrations of other ions are 126 mM (chloride), 5 mM (potassium), and 2 to 75 mM (bicarbonate) (increasing toward ileum), with a corresponding mean ionic strength of 0.14 M (7,23). Despite the dissolved electrolytes and the pancreatic and biliary secretions, the fasted state intestinal fluid appears to be hypoosmotic. Osmolality values between 140 and 270 mOsm/kg have been recorded (7,10,21).

Recently, the volume and distribution of the intestinal fluid in the gut have been discussed. Using magnetic resonance imaging it has been shown that volume of intestinal fluid in fasted persons is highly variable, ranging from 45 to 320 mL (24), comparable with postmortem fluid volumes of about 60 to 350 mL (25). After intake of food, the volume of "free fluid" is decreased to 20 to 160 mL, accompanied by increased filling of the proximal small intestine by a slurry of ingested food.

Furthermore, the intestinal fluid is not homogeneously distributed along the gut, but forms fluid pockets. In the fasting state, the number of fluid pockets ranges between two and eight with a median free fluid volume per pocket of 12 mL. In the fed state, the number of free fluid pockets increases but the median free fluid volume per pocket decreases to only 4 mL. One important consequence of the distribution of free fluid into pockets is that solid dosage forms that reach the intestine may only be partially or not at all surrounded by free intestinal fluid. The lack of contact with a dissolution medium in the intestine may cause variable in vivo dissolution and absorption. This may be even more pronounced in the fed state where the free fluid volume is decreased and the contact with the ingested food slurry may cause larger in vivo dissolution variability.

Small Intestine—Motility and Transit

The standard textbook rule stating that the small intestinal transit time is three to five hours is generally correct. However, one should be aware that the transit may occasionally be significantly faster or slower. Small intestinal transit times of approximately one hour as well as more than six hours have been observed (14). In healthy persons the transit through the small intestine does not seem to be influenced by the physical state, or the size of the dosage form, nor the intake of food (14).

The periodic muscular contractions in the wall of the small intestine achieve two objectives: local stirring to bring the dissolved luminal content close to the intestinal wall and propulsion of material toward the distal intestine. This is accomplished by a combination of annular constricting activity and peristaltic movements. Imaging techniques have shown that the transit of solid material through the small intestine is characterized by series of fast movements intervened by periods of stasis (26). These movements become weaker toward the distal small intestine.

COLON

Role and General Description of the Colon

In the colon, the main activities that can be relevant to drug release and absorption are fermentation by the large bacterial population, reabsorption of water and electrolytes and subsequent formation of the stools, and elimination of waste materials with defecation.

The digestive functions of the human colon are mediated by large populations of anaerobic bacteria. Upon entering colon the amount of bacteria per milliliter increases several orders of magnitude to about 10^{10} to 10^{12} /mL. The principal substrates of the bacteria are dietary residues that have escaped digestion or absorption in the small intestine, such as dietary fiber (typically indigestible celluloses and cellulose derivatives) and certain sugars and carbohydrates that are either not digested in the small intestine or have not been completely absorbed there. The end products of the bacterial fermentation reactions in colon are low-molecular-weight carboxylic acids, usually referred to as short-chain fatty acids (SCFAs), and gases such as carbon dioxide. The most common SCFAs in colon are acetic, propionic, and butyric acids with a total concentration adding up to approximately 120 mmol/kg colonic content (27). Other acids, for example, valeric and lactic acids, are also formed.

With respect to conservation of water and electrolytes, the colon is able to act as a low-capacity but high-efficiency site of reabsorption, with only about 200 mL of water (mostly bound to the stool constituents) being eliminated with each passing of stools. Electrolyte and water reabsorption are closely coordinated. In addition to absorbing these substances, the colonic mucosa is also able to absorb the SCFAs and bile salts that have been deconjugated by the bacteria. However, if the concentration of bile salt entering the colon is too high, a watery diarrhea would result (this can happen, e.g., in short bowel syndrome).

With respect to drug absorption, the colon offers fewer and less efficient absorption mechanisms than the small intestine. Active transport (except for a few electrolytes) mechanisms appear to be lacking in the proximal colon. The paracellular pathway is also highly restricted in the colon, as the gaps between the cells (tight junctions) become considerably tighter in the colon and only the smallest molecules (probably molecular weight 60 or lower) can be taken up by this route. So, typically compounds that are primarily absorbed by one of these mechanisms will be very poor candidates for traditional modified release formulations that should release the drug over eight or more hours in the fasted state. As the membrane of the cells lining the colon is a lipid double layer, analogous to the apical membrane of the absorptive cells in the small intestine, the transcellular passive diffusion mechanism is still available. Because the proximal colon is much shorter than the small intestine and its mucosa lacks villi, the effective surface area available for passive transcellular diffusion is comparatively much lower than in the small intestine. This lower surface area is offset at least partly by the longer residence time in the proximal colon than in the small intestine, with the result that some compounds such as metoprolol show similar overall fraction absorbed values when infused into either region. Therefore, before embarking on a formulation drive to develop a once-a-day oral dosage form of a short half-life drug, it is imperative to understand the mechanism(s) by which the drug is absorbed across the gut wall.

Colon—Fluid Composition

Our knowledge about the colonic environment and the colonic fluid is limited compared to the more proximal parts of the GI tract. The methods for collecting the content of colon employed so far include postmortem acquisition from patients with colostomy, during colon resection or during colonoscopy.

As a result of the formation of SCFAs by fermentation, the pH falls considerably from the terminal ileum to the ascending colon. A number of investigations have shown that pH in the caecum/ascending colon is on average just over 6, but values between 5.7 and 8.4 have also been reported (19,28,29). The SCFAs are quickly absorbed and metabolized during transit through the proximal colon, and in concert with bicarbonate secretion result in a rise in the luminal pH toward the distal colon. The rare observations of pH in descending colon/rectum point toward a value closer to 7.

The buffer capacity of colonic fluid collected from caecum/ascending colon appears to be higher than in the small intestine, most likely because of the presence of the SCFAs in this region. The buffer capacity toward acid challenge has been determined to be approximately 20 to 40 mmol/L $\times\Delta$ pH and toward base challenge approximately 10 to 20 mmol/L $\times\Delta$ pH (28,29).

The majority of the bile acids that reach the ileum are absorbed by the ileal bile acid transporters. The minor fraction of bile acids that escape the enterohepatic circulation and enter colon are metabolized by the bacterial flora. First, deconjugation takes place in which glycine or taurine is removed. Second, dehydroxylation of the sterol structure transforms primary bile acids to secondary bile acids. The solubility of many of the deconjugated bile acids is quite poor and they cannot be reabsorbed. Consequently, the bile acid concentration in colon fluid is low and most of the bile acids entering colon are excreted in the stool. The total content of dissolved bile acids in colonic fluid collected during colonoscopy varies between 10 and 1000 μM (28,29).

In spite of the reduced concentrations of bile acids compared to the small intestine, the surface tension of the colonic fluid remains relatively low. On average, the surface tension is approximately 40 mN/m (28,29).

The colonic fluid osmolality may be of potential importance for performance of modified release formulations based on osmotic pumping and for hydrophilic polymer-based formulations. Reported values range from 30 to 350 mOsm, indicating that osmolality in this region may deviate in both directions from the accepted isoosmotic value of 270 mOsm (Table 1). The fact that SCFAs are primarily formed as a result of bacterial activity on *dietary* residues for the colonic environment suggests that there may be differences in osmolality as well as pH and buffer capacity between the fed and fasted state. Such differences have also been observed experimentally (28,29).

The mean total fluid volume in the ascending colon has been determined by a scintigraphic method to average 162 mL, with individual volumes ranging between 82 and 303 mL (30). Using magnetic resonance imaging, the total volume of free water in colon was estimated to be less than 20 mL, with individual values between 1 and 100 mL (24). Postmortem fluid volumes in colon varied significantly, with values reported between 7 and 430 mL (25). For comparison, the volume of colonic contents collected during colonoscopy with approximately 10 minute effective collection time was approximately 25 mL with a "free" water content of 60% to 70% (28,29). It has recently been observed that the chyme remnants entering the colon through the ileocecal valve are much more viscous than plain water. Thus, it is not surprising that the amount of free water, that is water not bound to the solids that will form the stool (meal

TABLE 1 Physiological Factors Potentially Influencing the In Vivo Performance of Modified Release Formulations, A Comparison of Small Intestine and Colon

Physiological factor		Small intestine		Colon	
pH	Fasted state	Proximal	6.5 (2–7.5)	Ascending	6.2 (5.7–7.8)
		Distal	7.5	Descending	7
	Fed state	Proximal	5.5	Ascending	6.0
Buffer capacity (mmol/L $\times \Delta\text{pH}$)	Fasted state	2–6		20–30	
	Fed state	13–30		40	
Osmolality (mOsm/kg)		140–270		30–350	
Surface tension (mN/m)		30		40	
Bile acids (mM)	Fasted state	2–6		0.01–1	
	Fed state	4–37		0.01–1	
Free water volume (mL)		20–350		1–400	

remnants, sloughed off cells, and nonviable bacteria), is low in the colon. Additionally, as already pointed out, the ascending colon is very efficient in absorbing water and electrolytes, resulting in even lower availability of free water in transverse and descending colon. In line with the observations for the small intestine, the free water in these regions appears to be distributed in small separate pockets containing only about 1 to 2 mL water apiece (24). The lack of free water is one reason why drug absorption from the transverse and descending colon is usually inconsequential after an oral dose. The second reason relates to the different motility patterns in the proximal versus the lower parts of the colon.

Colon—Motility and Transit

The total colonic residence time is often quoted at around 24 hours, but the variability is high. In contrast to the small intestine, the colonic residence time for small pellets tends to be longer than for larger units such as tablets (31). A mean colonic residence time of 28 hours (range 6–48 hours) has been reported for a pellet formulation, while in the same study the mean residence time for a tablet was 15 hours (range 3.8–26 hours) (15). Variability in colonic transit time is caused by a number of factors. First, particle and fluid movement in the proximal colon can occur in both directions, which can be so pronounced that the order of arrival at the end of this segment may not correspond to the order in which the fluid/particles entered the colon. Second, there is a so-called gastrocolic reflex, which frees the colon (starting at about the right flexure, i.e., transition point from the proximal to the transverse colon) of its contents in response to meals in the course of a few minutes. Further, this reflex does not always occur in response to all meals, but rather seems to be strongest with intake of the first meal of the day, and also seems to be more pronounced in some individuals than in others. So it is wise to consider colon transit in two stages: passage through the proximal colon (which typically takes 5–12 hours) and passage through the rest of the colon (from a few minutes to days), bearing in mind that the viscosity of the luminal contents and free water volume are sufficient only in the proximal colon with respect to capacity for drug release and absorption.

SUMMARY OF GI PHYSIOLOGY AS IT PERTAINS TO DRUG RELEASE AND ABSORPTION

In this chapter, we have tried to provide an overview of the important factors to drug release and absorption from the GI lumen. These include the fluid composition in the various segments of the GI tract (important to release and stability of the drug), the residence times in each segment (“time allowed” for release and absorption), the importance of the segment to drug absorption in general, and the mechanisms of absorption available to the drug in each segment (efficiency of absorption). With these considerations as a background, we now invite the reader to explore the details of drug delivery to the GI tract in the next chapters, as well as to become familiar with some of the key methodologies used to characterize the release and absorption phenomena and the pharmacokinetic ramifications of administering drugs orally.

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Drug Transport Mechanisms Across the Intestinal Epithelium

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INTRODUCTION

The most convenient and used route of administration of drugs for targets within the systemic circulation is oral administration. To obtain a successful oral formulation, the drug has to have properties for entering the circulation in amounts sufficient to produce a therapeutic effect. Low oral availability of a drug is not only a problem for exposure and lack of efficacy but is also often associated with significant variability, both between different treatment occasions and between different patients/individuals. In addition, from a delivery perspective, drugs with problematic absorption characteristics are also difficult to combine with an appropriate formulation, since changes in the formulation behavior might not always result in improved absorbability. Knowledge of the reasons for low and variable absorption/bioavailability is, therefore, of importance for efficacy and to be able to select the candidate with the most appropriate properties for development, ensuring fast and efficient progress in clinical studies.

Oral bioavailability $F(\%)$ of a drug is determined by the fraction of the oral dose of the parent compound available in the systemic circulation *after* extraction, both by intestinal membranes *and* the liver. Additionally, it can be explained as the extent to which the active moiety is absorbed from a dosage form *and* becomes available in the systemic circulation. By definition, $F\%$ is, therefore, different from the percentage of the oral dose absorbed, which is defined as the fraction of the oral dose absorbed across the intestinal membrane (f_a) *before* entering the liver (1). f_a does not take a potential loss of the parent compound by metabolism into account, but reflects rather the total fraction of the dose crossing the first apical membrane of the enterocytes, including both parent and possible metabolites (2).

The formula $F = f_a \times f_g \times f_h$ is a well-documented way of explaining components of oral availability (1), where F is the bioavailability of the parent drug in the systemic circulation, for example, the fraction escaping both gut and liver extraction; f_a is the fraction of the oral dose absorbed, and is the total fraction of drug and its metabolites appearing in the body (by mass balance), f_g is the fraction escaping gut metabolism, and f_h the fraction escaping liver metabolism. If a drug is 100% absorbed ($f_a = 1$), but is metabolized in the gut wall to an extent of 50% ($f_g = 0.5$), and in the liver to 50% ($f_h = 0.5$), the oral bioavailability of the parent drug is only 25% ($F = 1.0 \times 0.5 \times 0.5$). Similarly, if a drug is only 50% absorbed and has the same f_g and f_h as the previous molecule, the oral bioavailability of that compound is as low as $F = 0.5 \times 0.5 \times 0.5 = 12.5\%$. This means that basic requirements for a drug molecule to obtain sufficient oral absorption, such as luminal solubility, permeability, and metabolic stability, are factors that need to be optimized during drug discovery to achieve sufficient exposure.

Processes of drug absorption after oral administration

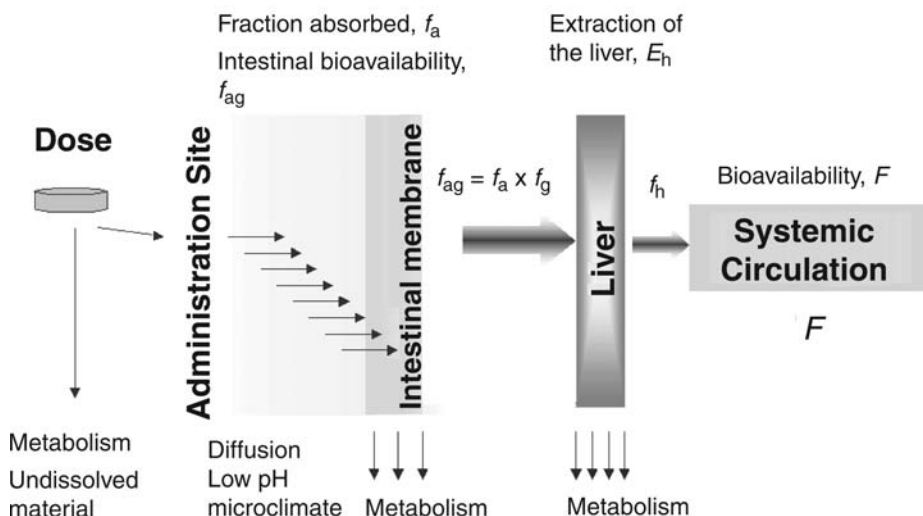


FIGURE 1 Illustration of the different processes involved in drug absorption from site of administration to bioavailable drug in the systemic circulation. Arrows in the intestinal lumen indicate part of the administered drug will diffuse toward the intestinal membrane and the part of the administered dose will, which is degraded or undissolved, not be available for absorption. Arrows below the intestinal membrane and the liver indicate the extraction (i.e., metabolism) in these organs. *Abbreviations:* f_a , fraction of the oral dose absorbed across the intestinal membranes; f_{ag} , available fraction in the portal vein after a possible extraction in the gut membrane (due to metabolism); f_g , fraction of the oral dose escaping gut metabolism; f_h , fraction of the oral dose escaping hepatic metabolism; F , total fraction of bioavailable drug in the systemic circulation after the possible extraction in the liver; E_h , extraction of the liver.

The formula forms a basic understanding of the different processes involved after oral administration, and can be used to simplify the thinking of where the drug may be lost and potential requirements for optimization. A simplistic view of this has been illustrated by many authors (1,3–5), and is shown in Figure 1.

Several processes are involved during the absorption process of a drug. Processes preceding the actual movement through the lipid bilayer structure, for example, dissolution/release from formulation, affecting the drug concentration at the site of absorption, and the processes of crossing the intestinal membrane into the body compartment. It has earlier been suggested that factors involved can be divided into three groups, and there are different suggestions for the grouping of these factors in the literature (2,6–9). If taking into account the physiology of the intestine, the structural properties of the drug molecules and the dynamic that occurs along the gastrointestinal (GI) tract, factors can be divided into the following: (i) those factors representing the chemical structure and property of the drug molecule, physicochemical factors, drug solubility, partition coefficient ($\log D$, $\log P$), pK_a , molecular weight/volume, aggregates, and particle size; (ii) factors relating to the content of the luminal fluid, such as

pH, bile, and food, affecting the drug concentration at the site of absorption; and (iii) factors like the anatomy and physiology of the intestinal membranes, for example, absorptive surface area, blood flow, membrane permeability, cellular enzymes, and transporters (2,6,7). The interplay between the factors should always be kept in mind when using different *in vitro* and *in vivo* models to predict drug absorption in humans, since an *in vitro* system is a typically static system and may very well lack the proper intestinal milieu, dynamics, and changes that can occur *in vivo*. During screening for structure property relationships (SPR) for drug transport in the early phases of drug discovery and development, the intestinal membrane and milieu are naturally kept constant (the use of one screening model, for example, human colon adenocarcinoma (Caco-2) monolayer and one pH buffer system (i.e., pH 6.5), and the drug characteristics are varied by testing multiple series of compounds with a variety of physicochemical properties.

Regionally within the GI tract both the properties of the intestinal membranes, that is, lipid composition, surface area, protein content of transporters and enzymes, as well as the components and pH of the intestinal luminal fluid change (10–13). Hence, the absorption environment of a drug (with respect to both luminal and mucosal aspects) may well change with region (14–20), a process very difficult to mimic in a static *in vitro* system. Apart from permeability of the intestine to the drug molecule, the time the molecule spends in the region of absorption, that is, transit time, is also of great importance. Generally, transit times in humans are in the order of seconds in the esophagus, 0.5 to 1.5 hours in the stomach, 3 to 4 hours in the small intestine, and 8 to 72 hours in the colon (21). This means that a long transit time in a specific region can compensate for a low permeability, but will be a negative factor for metabolic stability.

Therefore, more complex methods like *in vivo/ex vivo* animal studies are frequently used in parallel to *in vitro* experiments and modeling to provide more insight in mechanisms, get an integrated view of the level of oral drug absorption in humans, and thus obtain better predictions.

This chapter offers an overview over mechanisms of transport across an intestinal membrane, descriptions of processes involved, and some techniques used to obtain information for good prediction of human drug absorption.

DETAILING PROCESSES OF TRANSPORT ACROSS INTESTINAL MEMBRANES

The concept of drug absorption needs to be well defined, that is, definitions need to be clarified and possible factors influencing the data stated clearly. To simplify, the terms used in this chapter are, therefore, presented here.

Oral drug absorption can be seen as a combination of processes. Therefore, oral drug absorption contains information on both, extent and rate of absorption as well as on luminal events such as dissolution and degradation that affect the effective concentration at the site of absorption.

When looking at intestinal absorption in an attempt to estimate or predict the f_a in humans, one should be aware of that there are no *in vitro* methods that, at present, can give values for f_a and f_g separately. The techniques presently available in the literature give data and information on either disappearance from luminal solution or appearance on the other side of an intestinal membrane (6,7). The concentration of the drug in the compartment analyzed is a

result of several processes occurring before and during the analysis. The intestinal membrane, that is, the enterocytic cells, and intestinal lumen contain enzymes, and thus, transport from the lumen across the segment or epithelial layer describes the product $f_a \times f_g$ and can be further defined as the intestinal bioavailability, f_{ag} (2).

The factor, f_a , can be estimated by the determination of drug permeability and solubility in the biological fluid, and is often expressed by the use of Fick's first law [eq. (1)]:

$$J = Pe(C_2 - C_1)xA \quad (1)$$

Where J is the flux of molecules crossing the unit area of mucosal surface A , Pe is the ability of the membrane to let the compound through, and $(C_2 - C_1)$ is the concentration difference between the two sides of the intestinal membrane. Since the number of molecules crossing the membrane is dependent on the concentration gradient, there is a strong relationship between the flux, J , and the soluble and free fraction of the compound in the gut lumen.

The apparent permeability (P_{app}) of the intestinal membrane can be calculated using equation (2):

$$P_{app} = \frac{(dQ/dt)}{(A \times C_{d0})} \quad (2)$$

where dQ/dt is the rate of appearance of drug on the receiver side of a membrane, C_{d0} is the initial drug concentration on the donor side, and A is the surface area of the filter membrane.

The amount of drug that can be absorbed is dependent on many factors, for example, concentration in the gut lumen, which, in turn, is both related to the dose administered, the solubility of the drug in the intestinal fluids, the volume and composition of the fluid (13,22,23), pH (for ionizable drugs), and on potential binding or degradation in the luminal fluid (7,24,25). Complete absorption can be said to occur when the drug has maximum permeability coefficient and maximum solubility at the site of absorption (26). Thus, to obtain complete absorption of a drug, the molecular form must be both, adequately water soluble and lipid soluble, to be able to penetrate the lipophilic core of the enterocytic plasma membrane (4,27).

Fick's first law is only applicable for passive diffusion, both transcellular and paracellular transport, assuming no metabolism occurs. However, if carrier-mediated transport (see section below) or metabolism (section below) is to be taken into account, fluxes of molecules across the membrane will have a different dependency on the concentration gradient. To describe the total process, Fick's first law [eq. (1)] is combined with a Michaelis-Menten equation obtaining equation (3):

$$J = P(C_2 - C_1) + \frac{(T_{max} * C)}{(K_m + C)} \quad (3)$$

where the Fick's first law is combined with T_{max} , the maximum rate of the substrate transported via the transporter across the membrane, K_m , the concentration at half of the maximum transport rate (i.e., affinity), and C , the concentration of the substrate at the site of the transporter, preferably expressed as the unbound concentration.

If the compound is transported via a transporter in the opposite direction against the passive diffusion and/or out of the cell, this process can be indicated by a negative sign before the active transport part, that is, the *M-M* part, of the equation [eq. (4)]:

$$J = P(C_2 - C_1) - \frac{(T_{\max} * C)}{(K_m + C)} \quad (4)$$

For uptake, transporters favoring transport in the absorptive direction, concentration above the K_m of the substrate of the compound in the luminal fluid will potentially saturate the transporter, inducing nonlinearity in absorption with dose. This will lead to lower f_a with higher dose. By contrast, if an efflux transporter is involved favoring secretion of the compound back into the gut lumen, an increased concentration in the luminal fluid will saturate the secretory part and increase the number of molecules reaching the portal vein. In this case, the dose nonlinearity results in higher f_a with higher dose. If several transporters are involved in the transport across the membrane, the total transport characteristics will be dependent on the sum of the kinetics of each of the individual transport processes.

First-pass metabolism in the enterocyte causes dependency on the concentration at the site of absorption similar to that observed for transporters and follows, in general, *M-M* kinetics, with kinetics dependent on the sum of each the individual enzyme involved. At low luminal concentration (below K_m of the enzyme-substrate interaction) the enzymatic degradation can be dominant, resulting in lower mass transfer of the compound crossing the membrane than would occur if metabolism were not involved.

All active processes in the intestinal membranes are dependent directly or indirectly on ATP and often linked to ion transport such as Na^+ or H^+ , and enzymatic processes are similarly dependent on the generative ability of the membrane to yield cofactors such as NADPH (4,28). This is important to bear in mind when setting up in vitro systems to mimic the in vivo transport, in that conditions in vitro need to be optimized with respect to pH, specific ion concentration gradient, and generation of NADPH to give the best data for prediction.

DRUG TRANSPORT MECHANISMS AND THEIR REQUIREMENTS

Drugs permeate the intestinal membrane not only by passive diffusion but also by multiple and parallel processes. Passive diffusion can occur transcellularly across the lipid membrane or paracellularly between the epithelial cells through water-filled pores in the tight junctional complex (Fig. 2).

The transcellular route can also be transversed via carrier-mediated processes by the use of transporter proteins, favoring influx into or efflux out of the epithelial cell (4,6,7,29–31).

The molecular properties resulting in transport via carriers are in most cases completely different from that favoring simple-passive diffusion. For instance, the passive transcellular diffusion is often guided by partitioning into the lipid bilayer membrane, which is characterized by well-known properties, such as lipophilicity, polar surface area (PSA), hydrogen bonding potential, nonpolar surface area (NPSA), and number of rotatable bonds (32–38). Since the

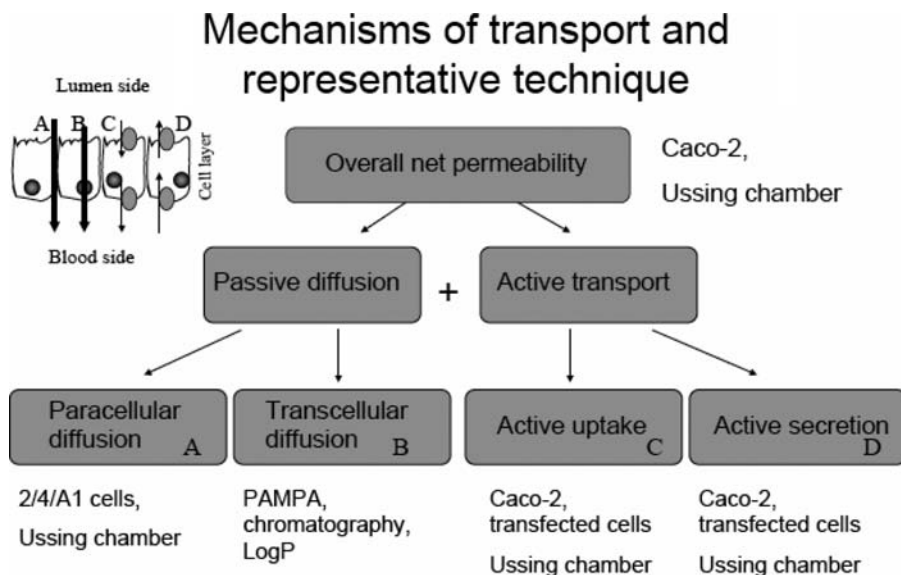


FIGURE 2 Illustration shows the different mechanisms involved in transport across the intestinal membrane and examples of methods that can be used for evaluation of the specific mechanisms. Insert drawing shows the pathways for a drug when crossing the epithelium. Source: From Ref. 31.

passive transcellular route is considered important, Lipinski's "rule of five" has been extensively utilized as a rule of thumb when large libraries of compounds need to be evaluated (38). This simple rule states that good f_a can be achieved if the molecule contains less than 5 hydrogen donors, less than 10 hydrogen acceptors, a molecular weight less than 500, and a $\log P < 5$. Other techniques representing the passive transcellular permeability rely on calculations of multiple molecular properties of different complexity, for example, $\text{clog } P$, dynamic averages of PSA and NPSA, partitioned total surface area, hydrogen bonding capacity/strength, number of hydrogen bonds, polarisability, integrity moments, solvation properties, and charge (32–38). During the early phases of drug discovery these *in silico* techniques are important since they offer data on permeability and solubility without the need for synthesis of compounds. Additionally, experimental determination of $\log D$, $\log P$, or chromatographic measurements and capillary electrophoresis can be used to get an estimate of the passive transcellular transport across a membrane. These techniques represent very simple systems well suited for high throughput, use only small amounts of compounds, and have all been found to correlate relatively well with Caco-2 permeability or $f_a\%$ (31,39–44). Artificial membranes (PAMPA), consisting of an artificial membrane lipid layer made of mixtures of lecithin or membrane phospholipids and inert organic solvents on a permeable support, have also been developed (45). PAMPA is based on a 96-well format, well suited for high throughput, and correlates relatively well to data from the Caco-2 cell monolayers, rat *in situ* perfusion (46), and human fraction absorbed or permeability *in vivo* (47,48).

In contrast to passive diffusion through the lipid bilayer, the aqueous pathway of the paracellular route is governed by molecular weight/volume, flexibility, and charge (49–51). Transport across this route is thought to contribute very little to the overall transport of drugs, since the surface area of the tight junctional pathway constitutes less than 0.1% of the intestinal membrane and is restricted to small polar compounds ($MW < 250\text{g/mol}$; $\log D < 0$) (49,51). However, this statement might be biased by the fact that many drugs on the market and in the pipeline are often of higher MW and rather hydrophobic. Matsson et al. (40) showed recently that there might be an underestimation of this route for polar small molecules. In addition, the popular screening techniques for oral drug permeability, for example, the Caco-2 cells, seem to underestimate the transport much more than do other techniques such as the rat duodenal cell line 2/4/A1 cells (40) or the excised intestinal segment or perfusions (17,49,52,53). If the paracellular route is to be utilized, screening should be done in relevant models, such as the 2/4/A1 cell line, perfused animal intestinal segments or the Ussing chambers. It should, however, be noted that paracellular transport seems also to be species dependent, with differences between rat, dog, and human *in vivo* data. These differences need to be taken into account for data interpretation (53,54).

Structural requirements for carrier-mediated processes depend on binding of the compound to the carrier (affinity) and translocation, and, thus, are driven by the size and structure of a transporter pharmacophore in the molecule. Several attempts have been made to determine potential structure-activity relationship (SAR) for substrates or interaction to transporters, ABC transporters (ATP-binding cassette family, that is, MDR1, as well as for uptake transporters PepT1, OCTs, etc. (55–65). If the compound is a substrate to an efflux transporter, the effective permeability across the membrane, it can be less than, would be predicted from the physicochemical properties alone. Similarly, if an influx transporter is involved in the transport across a membrane, then the permeability measured is often higher than expected for these properties.

Compounds can also be transported across an epithelial membrane via endocytosis. This pathway is minor, quantitatively speaking, and is usually proposed for larger molecules such as proteins, antibodies, and hormones. This route has been of less interest for several years; however, with the larger interest in biologicals for therapeutic medicines, interest has been rekindled (66). Specific receptor-mediated endocytosis can even increase the oral availability of large peptides or antibodies compared with the level observed without using the endocytotic system (66).

ACTIVE TRANSPORT ACROSS THE INTESTINAL MEMBRANES

During the last decade it has become evident that many drugs possess structural properties suitable for carrier-mediated transport, either as influx or efflux, out from the cell or body. Examples include some β -blockers, ACE inhibitors, β -lactam antibiotics, statins, and prodrugs of antiviral agents (51,67–78). Efflux proteins, belonging to the ATP-binding cassette family of proteins, are known to carry the drug from the inside of the cell back into the intestinal lumen, for example, MDR1 (ABCB1), BCRP (ABCG2), and MRP2 (ABCC2). These, nowadays, well-known transporter systems are proposed to limit the overall absorption of many drugs in the GI tract (18,30,68,69,73,78–80). The efficiency of

the transport via these proteins is guided by the intracellular free concentration of the substrate. This means that the substrate to the transporter either has to possess passive or active transport properties, good enough to enter the cell or it has to be formed inside the cell. For determination of affinity to these efflux transporters, knowledge of the true concentration at the site of the transporter is important, that is, the intracellular fraction unbound. This parameter is hard to get at experimentally, although some efforts have been made to take the intracellular concentration into account for transport (81–83). Rapid uptake and low recovery from the experimental system can also be due to intracellular binding to lysosomal structures, especially for weak basic drugs (84). These factors clearly show the difficulties in interpreting data from experimental systems, since the applied concentration on the apical side is not the concentration the transporter is exposed to, when using cellular systems. Kinetic constants of substrates to transporters are more preferably performed using membrane vesicles and inside-out vesicles obtained from transporter-transfected membranes. Commercial systems are available from SOLVOTM (28).

Equally important for drug absorption in the gut is the carrier-mediated processes favoring transport in the absorptive direction, such as the oligopeptide transporter family (e.g., hPePT1; SLC15A1), the amino acid transporter families (SLC1; SLC6; SLC7 families), monocarboxylic transporter family (MCT; SLC16), organic anion transporter polypeptide families (OATP; SLC21 and OAT; SLC22), and organic cation transporter family (OCT; SLC22 and OCTN2) (29,51,68,81).

Transporter protein expression differs between the different organs of the body and, also according to the site, in the intestinal tract (68,78–80), and a careful check of in vivo correlation of the cellular systems used for screening of the permeability is necessary (80). A comprehensive review on gene expression of transporters in tissues and orthologues cells demonstrated that the rank order of expression of transporters in the small intestine is HPT1 > PepT1 > BCRP > MRP2 > MDR1 (80). In addition, there was no correlation between human jejunum and colon.

Efforts have been made to target influx transporters to obtain higher quantities in oral drug absorption for hydrophilic compounds with otherwise low passive membrane permeability and low f_a . Popular transporters, as targets in the GI tract for increasing oral availability, are the PePT1 (SLC15A1), IBAT (SLC10A2), and also MCT1 (SLC16A1) and PAT1 (SLC36A1), which can be targeted by directly affecting the structural affinity or via prodrug design (58,62,72,76,77,85–87).

The relative contribution of active and passive transport across intestinal membranes is variable between compounds and methods used, as well as animal species, depending on the relative protein expression level of different transporters, susceptibility (affinity) of the compound to be transported by certain transporters on the one hand, and the concentration and ion gradient applied on the other (51). Hilgendorf et al. (77) showed recently that there is no correlation between the expression of transporters in the rat intestine compared with the human. This is important information, since the rat is often used in preclinical studies as a model for kinetics in humans. However, whether organ expression differences between species also translates into differences in protein expression or more relevant functionality is not yet known.

The concentration dependency of the transport via transporters often creates confusion in results and interpretation of data when comparing several techniques. At low concentrations within the intestinal lumen, that is, for drugs with very high potencies and for drugs with low solubility, involvement of efflux transport processes may have larger impact on the effective transport than for compounds administered at high doses and for highly soluble compounds (see above).

In addition, the relative contribution between active and passive processes *in vitro* versus *in vivo*, due to differences in concentration applied, is also one important concern for correct interpretation of *in vitro* compared with *in vivo* data. Intraluminal concentrations of a drug compound is usually much higher in the *in vivo* experiment (mM range), than screened in an *in vitro* system, for example, Caco-2 cells (μ M range), even though the compound is diluted in the GI fluids after dosing (88,89). Comparison can, therefore, only result in correct interpretation if the same drug concentration is applied in both systems or if passive diffusion is the process of permeation. In view of this, it is important to understand the different factors influencing drug transport and potential transporter interaction when interpreting *in vitro* and *in vivo* data.

METABOLISM DURING TRANSPORT ACROSS THE INTESTINAL MEMBRANES

The intestinal membrane does not only contain carrier proteins but also enzymatic proteins. These enzymes belong to several different families; cytochrome P450, lipases/esterases, amidases/proteases, and conjugating enzymes glucuronidases and sulfotransferases (7,90–93). The clinical relevance of intestinal metabolism during absorption has been debated for years, and has now been suggested to have a larger impact than previously assumed (93,94). In general, luminal enzymes belong to the group of proteases, amidases, and the esterase (lipase) families (7). The presence of microbes in the lumen results in degradation pathways that are mainly reductive (substrates include nitro compounds, sulfoxides, corticoids, double bonds, and azo bonds), or hydrolytic (substrates include esters, amides, glucuronides, and glucosides), with *N*-dealkylations and deamination also possible (25). Luminal enzymes are found at their highest concentrations in the upper GI tract while the microbial enzymes exist in their highest levels in the colon (7, 25). The membrane-bound enzymes, for example, in the brush border also show a gradient along the GI tract. The main cytochrome P450 enzyme in the small intestine seems to be 3A4, 2D6, and 2C9 (4,90–94). In addition, uridine diphosphate glucuronosyltransferase (UGT) and sulfotransferases (SULT) enzymes have good activity in the human small and large intestine (90), with greater activity in the small intestine than colon (see chap. 4).

In contrast to human jejunum, the parent Caco-2 cell line used in drug discovery expresses low levels of CYP3A4, the most important enzyme in the human gut (4,51,95–97) as well as very little UGT (4), and higher levels of glutathione transferase (GST) (98), while a subclone, TC7, has much higher levels (99). Hence, prediction of intestinal bioavailability ($f_{ag} = f_a \times f_g$) for compounds that are metabolized during transport over the intestinal membrane *in vivo* in humans via CYP3A4 could be overestimated using the Caco-2 model using the parent clone. On the other hand, other enzymes such as peptidases, amidases, and carboxylesterases, are present in the Caco-2 cell line (51). The only caution to

be mentioned is that the main carboxylesterase present in the Caco-2 is more similar to the human liver carboxylesterase hCE1 than intestinal hCE2 (100).

Differences in metabolism between intestinal models used, as well as species differences in intestinal metabolic capacity, create a number of mis-predictions of human fraction absorbed, and should be evaluated carefully (93,96,97,101).

Compound entering the epithelial cell may not only be a direct substrate for efflux transporters but may also be a substrate for enzymatic degradation. The resulting metabolite can then be a substrate for a transport out of the cell (102,103). This concerted action between transporters and enzymes further improves the efficient processes present in the body that are intended to eliminate unwanted products. However, this phenomenon also adds to the complexity of the mechanisms involved crossing the membrane. It raises the need for knowledge of the rate-limiting step, that is, transporter and/or enzymatic degradation, factors that may be difficult to estimate accurately from experimental models. Thus, the prediction of human result can also be difficult. In such cases, data from several models in parallel and use of a simulation model that can integrate these data would be of great help to understand the rate-limiting step (see below).

IMPORTANT EXPERIMENTAL CONDITIONS AFFECTING DATA OUTCOME

Small changes as pH gradients, stirring conditions, sampling times, and concentrations used are some of the key factors that have a large impact on the transport rates of drugs in vitro and, thus, care should be taken with respect to optimizing conditions when studying drug absorption (67).

Regional changes in luminal pH between pH 5 and 8, and in the acidic microclimate, at the surface of the membrane (20,21,104), may influence drug solubility, drug release, and/or permeability at various extents, depending on the pK_a of the compound (7,105,106). The relationship to pH for solubility and permeability are in opposite directions. Weak bases will be less soluble, but absorbed more efficiently in the lower parts of the small intestine, where the pH is neutral to basic (107), and acids will be preferably less soluble but absorbed in the upper GI tract, where the pH is more acidic (106).

pH and media compositions, therefore, contribute largely to the outcome of data and, thus, influence their interpretation (22). pH will affect the proportion between the uncharged and charged species for ionizable compounds, with the uncharged species of the drug molecule having the highest permeability. A pH gradient of 6 to 6.5 on the apical side and pH 7.4 on the basolateral side is recommended for screening to obtain a more in vivo like permeability value (105–109). In addition, a non-pH gradient system should be used to discard false predictions of efflux of weak bases (105). If active uptake is to be evaluated, for example, for weak acids or for compounds taken up by proton-dependent mechanisms, then two different systems, one without and one with a pH gradient, should be used to obtain maximum information on passive and active drug transport (106). Interpretation can also be false because of experimental limitations in the in vitro models with respect to nonspecific binding, low solubility, and the lack of physiological relevance of the commonly used buffers (22). The luminal content of bile acids, lipids, and enzymes as well as ionic strength also varies with regions (7), and this will affect the free concentration of

the drug at the site of absorption. The incubation buffer used should mimic the composition of the luminal fluid, but not influence the permeability characteristics of the epithelial membrane or lipid bilayer, and the drug compound should be chemically and physically stable and sufficiently soluble. The use of additives in the media has, therefore, been suggested, and a comprehensive review of commonly used media can be found (22). Finally, *in vivo* drugs absorbed across the intestinal epithelium are immediately carried away by the portal blood, preserving the concentration gradient as driving force for drug transport, that is, sink conditions are maintained. *In vitro*, however, proteins are usually absent in the receiver media. Maintenance of sink conditions during the transport experiment can be achieved by, for instance, inclusion of serum albumin or repeatedly exchanging the receiver solution. Sink conditions will have a major impact, especially when studying active (efflux) transport mechanisms, by increasing the absorptive transport of the compound (110). Inclusion of surfactants in the media to increase solubility may also result in transporter inhibition, as has been reported for P-gp (111), thus could be misleading if this is not a part of the intended formulation.

PREDICTIONS AND SIMULATIONS OF ORAL DRUG ABSORPTION

There are several models for getting information and data around absorption properties of molecules. These models are on the basis of either physicochemical structure-based predictions or experimental biological methods, or a combination of experimental and physicochemical predictions using simulation models, for example, physiologically based pharmacokinetic (PBPK)-based models.

The structure-based prediction models, that is, *in silico* models, mentioned previously in this chapter are based on physicochemical and molecular properties of the drug molecule.

The ideal experimental biological absorption method for studying pharmacokinetic and biopharmaceutical properties of drugs, in general, needs to have all the physiological and biochemical properties of the true barrier as well as being easy to use. The method also needs to have low variability between experiments and should be unbiased by the experimentalist.

The most popular methods for screening of intestinal permeability are the two cell lines, Caco-2 cells and Madin-Darby canine kidney (MDCK) cells ((32,51,67,112) since the study capacity in these models is far much higher than for excised tissues from animals or *in vivo*. Caco-2 has also been used in an automated mode, both as high-throughput 96-well plates and medium-throughput 24-well plate systems (51). Excised intestinal segments from animals or humans to be used as rings, sacs, or in the Ussing chamber, *in vitro* and *in situ* intestinal perfusions, *in vivo* cannulated or fistulated animals (6,7,51,113–119) are other methods that are used in parallel to cell lines to complement physicochemical knowledge and obtain a better understanding of drug absorption. *Ex vivo* methods can be used when the mechanisms of absorption (paracellular, transcellular, or carrier-mediated) and the enzymatic degradation or regional difference in permeability are to be evaluated, but these are of a much lower study capacity and throughput than cell lines like Caco-2.

Each of the *in vitro/ex vivo* techniques has been found to correlate relatively well with fraction of the oral dose absorbed in humans (32,117). It is very

important to experimentally determine a correlation to in vivo data on fraction absorbed, f_a , that is, do a validation curve, to completely understand the extent of absorption of a certain drug and the predictivity of the model (51). This is, however, not a simple evaluation since different methods represent different parts of the total absorption process, and the main barrier will have the largest contribution to the results. Data obtained from these models also reflects several barriers, that is, a mix of f_a and f_g (see above), and data confounded with metabolism is a common reason for deviations from a good correlation, since the appearance of the drug on the serosal side of the membrane preparation or cellular system will only show the fraction escaping metabolism in the cells (2).

Cell lines, transfected with human transporters, and human tissue-based ex vivo methods are, by definition, the most important for evaluation of transporter involvement and building of SPR because of the potential species differences in transporter substrate specificity (120). Transfected cells like membrane vesicles from Sf9 (insect cells) or frog (*Xenopus laevis*) oocytes, etc., are some of these systems now available (28). An increasing number of reports in the literature show the use of these transfected cells with one specific transporter/s, single or multiple, and with either the human or the animal variant (28,67,121–123), enabling detailed mechanistic evaluations. Investigations around the involvement of transporters in drug absorption, using these tools, can, however, overestimate the potential contribution of transporters to the overall drug absorption of the compound because of the overexpression of the transporter in the specific membrane. These tools are, therefore, more suited to aid the identification of a certain transporter involvement and determination of kinetic constants (K_m and T_{max}), but may not be suitable for estimation of the *quantitative* contribution or risk assessment of the overall oral drug absorption.

Also, commonly used preclinically is the prediction/estimation of f_a in humans for a particular drug estimated from bioavailability, F , measurements, and clearance (CL) obtained from evaluation of in vivo animal data, usually from the rat and/or dog (119). An estimate of f_a can be determined by taking into account the liver extraction (E_h) of the drug compound, $E_h = CL_h/Q_h$, and bioavailability, F , via the relationship, $F = f_a \times f_h$. An estimate of f_a can be obtained by the relationship:

$$f_a = \frac{F}{(1 - CL/Q_h)} \quad (5)$$

where CL means in vivo clearance and Q_h is the liver blood flow in a preclinical animal. The same relationship between CL_h and F in humans, as in the animal models, is assumed.

This way of estimating f_a does not take into account a potential intestinal metabolism or loss separately from the f_a estimate, thus the value is reflecting f_{ag} instead (as described above). If the f_a in several of the species, is similar then it is taken as being predictive of the data in humans. However, since a large variation in expression of transporters and enzymes between animals exist and since many of the new chemical entities developed will be given in lower doses (because of higher potencies), which increases the potential influence of transporters and enzymes, prediction of human f_a only on the basis of animal in vivo data might not be accurate. Knowledge of species differences is especially

important for scaling of in vitro or in vivo data from animals to humans (2,6,7,93,97,101).

Species differences in f_a are mainly due to differences in paracellular pathway, enzymatic degradation differences, both in the membrane and in the lumen, as well as transporter differences. The dog is well known to have less expression of P-gp (the MDR1 gene product) than humans or rats (124); thus, the limitation to transport by P-gp in this animal is less pronounced. The dog is also known to have higher fraction absorbed of polar compounds, indicating that the paracellular transport pathway is different and perhaps more accessible compared with humans (10). The larger size of the paracellular pathway in the dog intestine compared with other animals is confirmed by the oral availability of polyethylene glycol (PEG) molecules (54).

PBPK modeling and simulation of GI absorption are more and more frequently used to get an integrated view of multiple parameters involved in drug absorption as well as analysis and prediction of the plasma concentration–time profile in vivo (125–129). Such models can also evaluate the sensitivity to a certain parameter using sensitivity analysis, for example, sensitivity of changes in f_a to the solubility to obtain a feeling for the sensitivity of variation of the parameter tested (128,129). The PBPK models are generally based on a several compartment analysis of the intestinal absorption (e.g., an ACAT model) (125), which is based on a series of integrated differential equations to mimic the different regional events in the GI tract. PBPK models also take into account estimates of tissue partitioning in the organs for prediction of distribution. The models have values for several physiological parameters such as volumes, weights and blood flow rates to the body organs, radius of the intestine, transit times in different regions, and regional pH changes incorporated (128), and can be adopted to both pre-clinical animal species and to humans. The simulations use input data including physicochemical properties such as pK_a , lipophilicity and solubility, in vitro permeability such as from Caco-2, intrinsic metabolic stability $CL_{intliver}$, and can easily give information or hypothesis on what is the confounding parameter or rate-limiting step. Such models are available in the commercial software such as GastroPlus and SimCYP (130,131), and the literature also shows more specific and refined simulation models, such as those described in the work of Peters 2008 (128,129). (For more detailed information on PBPK models, see chap. 16.)

Many models in the literature do not take metabolism into account when the drug has to pass the intestinal membrane. Models that take into account metabolism in the gut include, for instance, the Q_{gut} model in the SimCYP software (130,131) and the intestinal loss parameter in the PBPK model, described by Peters (129). This model is basically based on the well-stirred model used for calculations of hepatic CL and is called the Q_{gut} [eq. (6)]. It uses a permeability term, CL_{perm} of the test compound, which can be obtained from permeability measurements using in vitro tools, like the Caco-2 or MDCK cells and a converting factor, and villus blood flow (Q_{villi}) to obtain CL_{int} in the gut describing gut first-pass F_g (131) equation (7).

$$Q_{gut} = \frac{Q_{villi} \times CL_{perm}}{Q_{villi} + CL_{perm}} \quad (6)$$

$$f_g = \frac{Q_{villi}}{Q_{villi} + CL_{intg} \times (1 + (Q_{villi}/CL_{perm}))} \quad (7)$$

Protein binding can be taken into account as for the well-stirred model to correct $CL_{int\ g}$ to unbound $CL_{int\ g}$. This way of calculating gut first pass has been questioned since physiologically the drug is not delivered via the systemic blood flow to the mucosa and protein binding, thus, cannot influence the movement of the drug from the lumen (93). A more simple, straightforward approach has been presented by Fagerholm, also incorporating the term of permeability, equation (8) (132).

$$E_{gut(compound)} = \frac{E_{gut(ref)} \times CL_{int(compound)}}{CL_{int(ref)}} \times f_a \quad (8)$$

Fagerholm uses a reference molecule, verapamil, with known extraction in the gut *in vivo*, instead of scaling factors, to convert *in vitro* CL_{int} to *in vivo* extraction in the gut (93,130–132).

Any of these models can be used for simulation and prediction of f_a and f_{ag} and the rate-limiting process/es during oral drug absorption, and will more or less give a correct description of the involvement of metabolism. If several of these models are used and support one hypothesis, the likelihood of a more accurate understanding is greater.

CONCLUSIONS

This chapter provides a short overview on mechanisms and processes involved in drug absorption. Knowing and measuring limiting factor/s and use of integrated models to optimize the predictions and to understand the importance of luminal concentrations, and regional differences in transporters and metabolism in drug absorption is important to prediction of oral absorption.

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Clive G. Wilson*University of Strathclyde, Glasgow, Scotland, U.K.***Werner Weitschies***Institute of Pharmacy, University of Greifswald, Greifswald, Germany***James Butler***GlaxoSmithKline R&D, Predictive Technologies, Essex, U.K.***INTRODUCTION**

The human gut has evolved over many thousands of years to provide an efficient system for the extraction of nutrients contained in a highly variable food supply. Within the mix of grain, meat, and berries, which formed the diet of ancestral mammals, poisonous seeds and berries were accidentally ingested and protective responses to the unwanted pharmacology were developed. Spitting out bitter materials and vomiting provided some level of protection for higher mammals, and intestinal mechanisms were developed to reduce exposure. Thus, the physiology of the digestive process is less than convenient for the efficient absorption of many of the modern therapeutic entities, which resemble such poisons. In addition, we differ in our genetic and social patterns, which in turn impact on the efficiency of absorption and clearance and, therefore, the time course of the effects that we see with medications. Variability in the plasma concentration-time profile within and between individuals can be strongly influenced by anatomical, physiological, physicochemical, and biochemical factors including nature of the mucosa, the available surface area, pH, and the presence of enzymes and bacteria. In particular, the influence of feeding and temporal patterns on gastrointestinal (GI) transit is of great relevance as a factor in the absorption of poorly soluble drugs.

A large body of published work on GI transit of formulations utilizing γ -scintigraphy appeared in the 1980s through this century (1), and the γ -camera remains a gold standard as an assessment method. Sophisticated γ -ray detecting camera systems and high-speed computer links enable the clinical investigator to image different regions of the body and to quantify organ function. Parallel developments have occurred in the field of radiopharmaceuticals, and a wide range of products are available that will exhibit uptake within specific tissues following parenteral administration. The situation with regard to investigations of GI transit is much simpler: the chief requirement is to be able to label different components within the formulation or food and for the label to remain associated with the component in both strongly acidic and neutral conditions. From the pharmaceutical perspective, the most important recent advances have come in the applications of other imaging modalities such as magnetic resonance imaging (MRI) and magnetic moment imaging (MMI), which are increasingly applied to help the pharmaceutical scientist to understand formulation behavior.

Functionally, the gut is divided into a preparative and primary storage region (mouth and stomach), a secretory and absorptive region (the midgut), a

water reclamation system (ascending colon), and finally a waste product storage system (the descending and sigmoid colon). The organization of the upper gut facilitates the controlled presentation of calories to the systemic circulation, allowing the replete person to perform physical work, to undergo social activities, and to go to sleep.

For conventional formulations, the important transit processes controlling tissue exposure are contact times in the various regions of the gut and the extent and nature of agitation. In addition to this, we must also consider the amount of fluid available and its composition. It is probably logical to consider the gut in appropriate sequence, as seen by a formulation, moving from mouth to anus.

ESOPHAGEAL TRANSIT

After the dosage form leaves the buccal cavity, which is a relatively benign environment, transit through the esophagus is normally complete within 15 seconds. However, this may be influenced by several factors, including the dosage form, exact mode of administration, posture, age, and certain pathologies (2,3). It has been known for many years that disorders of normal motility (dysphagia), left-sided heart enlargement, or stricture of the esophagus can result in impaired clearance of formulations, which, in turn, could result in damage to the esophageal tissues. Radiological studies of an asymptomatic group of 56 patients, mean age 83 years, showed that a normal pattern of swallowing was present in only 16% of individuals (4). Oral abnormalities, which included difficulty in controlling and delivering a bolus to the esophagus following ingestion, were noted in 63% of cases. Between 13% and 33% of patients have reported swallowing difficulties in nursing homes. Structural abnormalities capable of causing esophageal dysphagia include neoplasms, strictures, and diverticula, with several workers commenting that only minor changes of structure and function are associated specifically with aging. Nilsson and colleagues have developed a repetitive swallowing test, using a straw fitted with a pressure detector to measure suction pressure (5). The elderly group (mean age 76 years) was shown to have a slightly lower suction pressure than the younger control group (mean age 39 years), but more importantly, the length of time over which suction was sustained was significantly shorter. These data support the notion that the increased stiffness and lower muscle compliance occur in the elderly, who have little "swallowing reserve." The process of swallowing is usually followed by expiration of breath—in the elderly group 30% of the subject group ($n = 53$) inhaled immediately after swallowing and several of them developed coughing fits during prolonged swallowing maneuvers. This observation illustrates the complexity of information processing from local and central cortical systems and the manner in which respiratory and gustatory systems are controlled. Afferent information is more slowly processed in the elderly and deliberate maneuvers to compensate such as lengthened laryngeal vestibule closure might be useful (6). The difficulty for elderly patients appears to relate to neurological mechanisms associated with the coordination of tongue, oropharynx, and upper esophagus during a swallow. Diseases such as type 1 diabetes reduce the amplitude of peristaltic waves and further exacerbate the problems, particularly for solid swallows (7).

In scintigraphic measurements of transit rates of hard gelatin capsules and tablets, elderly subjects were frequently unable to clear the capsules (8,9). This appears to be due to the separation of the bolus of water and capsule in the oropharynx, resulting in a “dry” swallow. Capsule adherence occurred in the lower third of the esophagus, although subjects were unaware of sticking. The importance of buoyancy in capsule formulation has hitherto been ignored and may be an additional risk factor in dosing the elderly.

The issue of surface properties in tablets is also important and, surprisingly, small flat tablets can cause problems. In the development of a risedronate product, we needed to develop a procedure that was able to discriminate between alternative formulations. The key conditions necessary to differentiate among products with respect to the ease of swallowing was to dose the unit with one mouthful of water—30 mL. Using this procedure we demonstrated that small, uncoated, shallow convex-shaped tablets (9.5 mm diameter) were arrested in the esophagus more often than the final design of the formulation—an oval of $5.7 \times 11.5 \text{ mm}^2$ (2). In 5 out of 30 cases, esophageal transit of the smaller tablet was slower (10).

The elderly have fewer problems in clearing a liquid bolus compared to a solid mass, and thus it is common practice to crush medications for dysphagic patients. Scored tablets allow alternative approaches, and since there has never been a reported issue of over- or underdosing using this maneuver, it may be safer. However, van Santen and colleagues reported many instances in which scored tablets were physically not subdividable (11), suggesting that compendial leadership is needed on this issue.

GASTRIC EMPTYING AND RETENTION

Our understanding of the behavior of dosage forms in the stomach has been gained largely from scintigraphic studies in which solid and liquid phases of a meal and formulations are labeled with different radionuclides, most often technetium-99m (Tc-99m) and indium-111 (In-111) (12,13). These two radionuclides can be distinguished according to the energy of their emissions, and thus can be separately detected, even when both are present in the field of view.

Such studies have demonstrated that retention times of conventional formulations in the stomach are dependent on the size of the formulation (14). It has been reported in the endoscopic literature that a 5-cm length $\times$ 2-cm diameter rigid object will not pass through the stomach (15,16). The second important factor is the intake of food that causes the pyloric sphincter to increase sphincter tone and the caliber of the pylori-duodenal junction to reduce. The increase gastric residence time after a meal is initiated to ensure a steady flow of calories to the small intestine and is thus proportional to calorie intake. The third factor is the physical dispersion in the gastric contents, which can asymmetrically distribute or be uniformly spread depending on the dosing regime (17).

Nondisintegrating forms such as enteric-coated tablets dosed on an empty stomach are generally emptied from the stomach quite rapidly (typically within 2 hours following ingestion), while after a heavy meal they may be retained for a considerable period of time—over 15 hours if the feeding cycle continues (18). This is due to the sieving function of the digesting stomach, preventing delivery

of poorly digested food particles and other large objects to the duodenum. In contrast to common belief, there is no fixed caliber for particle retention during digestion. The relative retention depends on luminal factors including the viscosity of the gastric contents with the higher viscosity, allowing larger particles to be cleared (19).

Multiparticulate and disintegrating dosage forms will empty more slowly in the presence of food than in the fasted state. Since these dosage forms have the tendency to mix more or less evenly with the food, their entry into the small intestine will be strongly influenced by the calorific density and bulk of the ingested meal (14). The rate of gastric emptying, therefore, determines the absorption behavior, and it is reasonably reproducible. In contrast, the emptying of larger, nondisintegrating dosage forms and even small soft gelatin capsules is sometimes less predictable, and in these cases other nonradionuclide measurements may aid in the understanding of the dosage form behavior.

As an example, erratic performance of a soft gel formulation containing a poorly soluble drug was observed when given with a high carbohydrate meal (a baguette). Reduction of dose size increased the variability and there was some difficulty in the interpretation of these results using scintigraphy alone. It was necessary to utilize other imaging modalities, specifically MRI. Using this technique, the differences in proton shift of gut contents and tissues can be used to explore the behavior of formulations in the GI tract, provided that movement artifacts can be minimized. At first there were difficulties in obtaining good definition, until it was found that rolling the subject into a prone position immobilized the stomach contents: in this position the pressure of the viscera causes mixing to abruptly cease and the liquid and solid phases separate in the stomach.

The stasis produced by the maneuver allows the behavior of small objects to be clearly discriminated in the stomach, as illustrated in Figure 1 in which two filled gelatin capsules can be seen in the interpretation of these results using scintigraphy alone. It was necessary to utilize other imaging modalities, specifically MRI. Using this technique, the differences in proton shift of gut contents and tissues can be used to explore the behavior of formulations in the GI tract, provided that movement artifacts can be minimized. At first there were difficulties in obtaining good definition, until it was found that rolling the subject into a prone position immobilized the stomach contents: in this position the pressure of the viscera causes mixing to abruptly cease and the liquid and solid phases separate in the stomach.

Using this same maneuver, the MRI clearly revealed the heterogeneity in the stomach associated with the baguette-based meal and helped to explain the



FIGURE 1 Oil-filled gelatin capsules dissolving on the floor of the stomach. Subject is lying prone. The capsules can be seen as two bright objects in the liquid. Gas shows up black and the surrounding musculature is bright field. *Source:* From Ref. 20.

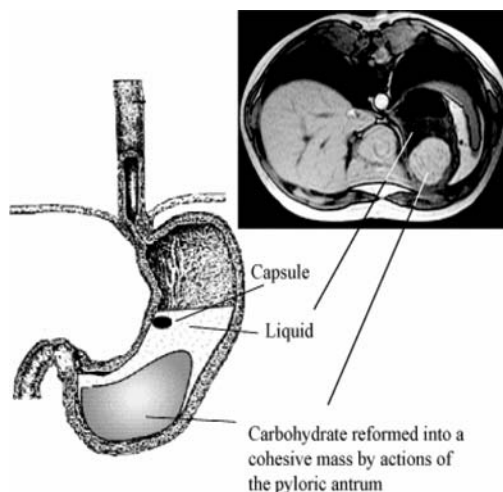


FIGURE 2 Magnetic resonance image showing the semisolid fraction of a sandwich-based meal lying in the stomach. A small capsule given soon after the meal floats on the liquid above the solid mass, becoming stuck in the gastric rugae in the body of the stomach or floats off ahead of the bulk of the gastric contents.

variability associated with the formulation. Figure 2 shows the semisolid fraction of a sandwich-based meal lying in the stomach.

Because the solid phase is not fully hydrated, it shows up as a bright doughnut-shaped solid against the liquid phase above it. Over a period of about 30 minutes to an hour, the solids gradually hydrate and the two phases are no longer distinct. It is well established that, after eating a meal, the shape of the stomach changes and the upper part (the fundus) relaxes to accommodate the extra volume. There is a short lag phase before the mixing movements in the lower part of the stomach (the pyloric antrum) increase. Accordingly, there is, therefore, a sharp contrast between the activity in the top and bottom parts of the stomach. During the early phase of digestion, the center of the lumen is relatively immobile and the secreted gastric juice flows around the food mass. This lack of homogeneity in the gastric contents after recent meal ingestion prevents efficient mixing and can have therapeutic consequences. For example, a small capsule given soon after the meal could either float on the liquid above the solid mass or float off ahead of the bulk of the gastric contents, resulting in quite different delivery patterns to the absorptive sites in the small intestine.

The lack of homogeneity after food also extends to both pH and hydrodynamics. Hila and coworkers (21) used a pH probe, moving stepwise upward through the stomach contents, to demonstrate that layers of low and high pH exist for about an hour following a meal consisting of chocolate milk and an egg McMuffin. Irrespective of body position, a more acidic layer with pH closer to that of the homogenous fasted state was detected both below and above a higher pH, food-buffered layer. Using a three-port pH ambulatory system, pH in the esophagus and stomach or the upper and lower stomach regions can be simultaneously monitored (1). These data show marked differences in the regions after a meal (see Fig. 3).

In a similar fashion, Simonian and coworkers (22) have demonstrated that pH is highly region dependent within the stomach. Their study used three breakfast types (bland, spicy, and fatty) to additionally show that these regional

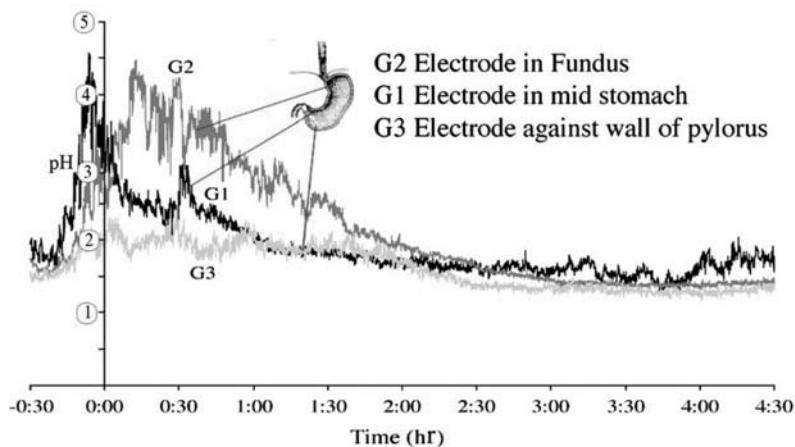


FIGURE 3 Variation in pH in the stomach after a meal measured in three regions simultaneously. *Source:* Adapted from Ref. 1.

pH differences, and length of time these differences were present, are dependent on the meal type.

In terms of hydrodynamics, there are marked differences in the forces a dosage form is likely to experience in the antrum (strong forces, high attrition) compared to the fundus (weaker forces, low attrition) and in the fed (higher attrition) versus fasted (lower attrition) state.

Such heterogeneity makes the environment experienced by a dosage form after dosing with food (and, potentially, drug released), highly dependent on location and residence time, particularly where drug release is pH and/or erosion dependent. In this context, the formulation of robust eroding matrices for modified release is a particular challenge. For instance, from *in vivo* studies comparing the pharmacokinetics of nifedipine once-daily formulations (23–25), it appears that gastric residence and hydrodynamics have a crucial role in drug release, and is the most likely cause for many of the significant intraformulation pharmacokinetic differences observed.

It is reasonable to expect that altering the balance between solids and liquids will affect emptying of both phases. The interaction is quite complex: Collins and coworkers tried increasing the volume of the solid phase relative to the liquid in meals containing either 100 or 400 g minced beef and a fixed amount of water. They showed that, with the larger meal, the lag phase increased from 31 to 56 minutes, but that after this lag time the emptying of solid was accelerated. Furthermore, the larger meal retarded intragastric distribution and gastric emptying of the liquid (26). On the basis of this observation, it would be expected that an oral formulation given after a large meal would show a decreased rate of emptying. Scintigraphic studies show that the tablet is generally held in the fundus and may remain static as in the upper stomach for more than an hour, as stirring movements are sluggish or even absent. In an imaging study (MMI) using magnetically labeled extended release hydrogel forming matrix tablets containing nifedipine, we observed that tablet intake after a meal resulted in a predominant location of the extended release tablets in the

region of the fundus. This resulted in low hydrodynamic stress because of low contractile activity of the fundus musculature and accumulation of drug substance in this region. This regional stasis leads to late maximal plasma concentrations ($T_{\max}$) and, in some cases, even dose-dumping-like peaks in the plasma concentration-time profiles that are related to sudden gastric emptying of accumulated drug substance (27).

Faas and coworkers (28) in Zurich were able to elucidate the cause of the observations made by Meyer and Lake (29), who showed a mismatch in delivery between the digestible fat fraction and the delivery of pancreatin from an enteric-coated pellet formulation. The study conducted by the Zurich group extended MRI observations on meal effects and homogeneity by studying meals that were homogenous, contained particulates, or were highly heterogeneous (a hamburger-based meal with different amounts of water). They showed that the intragastric distribution of the marker was highly affected by the consistency of the meal, whereas the amount of coingested liquid had a small effect. A large fraction of the contents of the fundus did not come in contact with the marker, and in agreement with our earlier studies (30), it appears that the liquid phase moved around the consolidated solid phase.

For certain drugs, it is desirable to increase the rate of gastric emptying to speed up absorption and achieve a faster onset of action. Grattan and colleagues reported that a novel acetaminophen (paracetamol) formulation containing sodium bicarbonate showed a shorter time to maximum serum concentration ($t_{\max}$), in both the fed and fasted states, compared to conventional paracetamol tablets (31,32). These results can be partially explained on the basis of an old observation of Hunt and Pathak, who described a prokinetic effect of sodium bicarbonate, which was maximal with an isotonic solution (33). Given that the recommended dose of the new formulation, two tablets taken with 100 mL water would produce an approximately isotonic solution of sodium bicarbonate, faster gastric emptying seemed a likely explanation for the faster absorption—at least in the fasted state. The new formulation was also shown to display faster *in vitro* dissolution compared to conventional tablets in 0.05M HCl, using the USP II paddle apparatus at low stirrer speeds (10–40 rpm). Although the reason for this faster *in vitro* dissolution remained to be established, it was proposed that there might be a corresponding increase in *in vivo* dissolution rate.

We suspected that the increased dissolution rate could be due to the altered hydrodynamic environment resulting from the release of gaseous carbon dioxide by the reaction of sodium bicarbonate with hydrochloric acid. According to the Noyes–Whitney equation, drug dissolution rate is inversely proportional to the thickness of the boundary diffusion layer at the surface of the tablet. Therefore, turbulence caused by gaseous carbon dioxide could effectively reduce the thickness of the diffusion layer and thus increase dissolution rate. To further investigate the influence of gaseous carbon dioxide on dissolution rate, our group carried out *in vitro* dissolution studies using carbonated and degassed soda water as dissolution media with a stirrer speed of 30 rpm. There was no significant difference between the dissolution profiles of the conventional formulation in the degassed medium and in 0.05M HCl. However, the carbonated medium increased the dissolution rate of the conventional formulation to such an extent that the dissolution profile was similar to that for the new formulation in 0.05M HCl. This is consistent with the hypothesis that the

increased dissolution rate of the new formulation in HCl is due to turbulence caused by the generation of gaseous carbon dioxide.

A combined scintigraphy and pharmacokinetic study was conducted in healthy volunteers, which allowed comparison of the *in vivo* rates of disintegration and gastric emptying with the serum concentration versus time profiles of the two formulations. Faster disintegration and gastric emptying of the new formulation was confirmed in both fed and fasted states, with the differences in gastric emptying being more pronounced in the fasted state and the differences in disintegration more pronounced in the fed state (34). As one might expect, the effect of food already present in the stomach appeared to impair the prokinetic effect of the sodium bicarbonate. Figure 4 shows representative scintigraphic images from an individual volunteer in the fasted state. After 5 minutes, the new tablets have largely disintegrated and some gastric emptying has already occurred, whereas the conventional tablets remain almost intact. After 60 minutes, gastric emptying of the new tablets is complete, while little emptying of the conventional tablets has occurred.

It has been established in many experiments that fat retards gastric emptying, although the presence of fat in the stomach is not the key issue. Much work has been done to establish the exact mechanism for this observation, and it has been known for many years that the fat effect is mediated through receptors

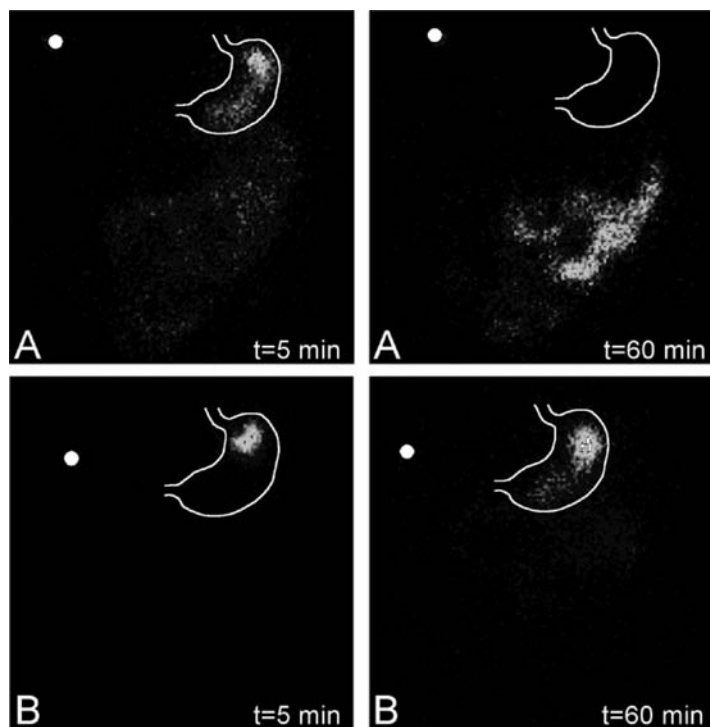


FIGURE 4 Representative scintigraphic images taken from a single volunteer following dosing with new paracetamol tablets containing sodium bicarbonate (A) and conventional tablets (B) in the fasted state.

in the small intestine (35). Studies in dogs using manometry and three-dimensional x-ray techniques established that the presence of fat in the upper intestine delays emptying by increasing resistance to flow through the pylorus (36). It has also been established that the hormone cholecystokinin (CCK) is at least partly responsible for this effect in humans (37). This leads to the possibility that fats could be used to retard the gastric emptying of drug formulations. Gröning and Heun incorporated fatty acid salts in formulations of riboflavin and nitrofurantoin and showed an increase in both gastric residence time and drug absorption (38,39). The effect of administering low doses of lipids (2 g) on gastric emptying of an intact matrix and on gall bladder contraction has been studied in man using γ -scintigraphy and sonography (40). Low volumes of long-chain lipids, but not medium-chain lipids, stimulated gall bladder contraction and elevated luminal bile salt and phospholipids, although the sampling technique probably underestimated concentrations. It was observed that the lipid caused the contents to halt in the jejunum beyond the sampling orifice of the duodenal catheter.

Modified release formulations with prolonged gastric residence time—so-called gastroretentive systems (41)—are of particular interest for drugs with poor absorption from deeper parts of the intestine as, for example, amoxicillin, ciprofloxacin, furosemide, and metformin. The challenge behind such drug delivery concepts is to assure complete drug release within the stomach. To avoid early gastric emptying, it is usually recommended to administer such gastroretentive drug delivery systems together with a meal (42–44). In case of the combination of clavulanic acid and amoxicillin as an extended release tablet with gastroretentive properties, it is furthermore required to administer the product at the beginning of a meal, as intake after a meal leads to reduced bioavailability of clavulanic acid caused by intragastric degradation of this unstable compound (45).

The emptying of the stomach may be incomplete and a mechanism controlling gastric emptying of residues accumulating in the stomach termed the migrating motor complex (MMC) can be recorded externally with electrodes on the abdomen. This has been extensively described in the literature following the first experiments described by Code and Martlett (46) and by Bull et al. (47). The strong contractile activity during phase III of the MMC is an important factor limiting the efficiency of gastroretentive dosage forms, and the vagaries in gastric emptying cause difficulties in interpretation of the efficiency of gastroretentive devices. It has been necessary to suggest clinical protocol designs to take account the effect of meal size and frequency on gastric emptying. Assuming that the phase III contractions of the stomach are the most challenging phenomena, the ability to resist two cycles of MMC would provide compelling evidence of effective gastroretention, as shown in Table 1.

The rationale is to reduce the possibility that an excessive food intake has occurred prior to arrival at the clinical site. It would be expected that two housekeeper waves (most likely at 10 a.m. and 1 p.m.) would occur between breakfast and lunch. The afternoon period rarely proves challenging, as the gastric activity subsides in the normal circadian rhythm. A successful gastroretentive dosage form should be able to survive at least two housekeeper waves, surviving into the post-lunch period (i.e., more than 5 hours). A normal meal at lunch would probably permit more than eight-hour retention. Finally, it is

TABLE 1 Suggested Meal Protocol for Testing Gastroretentive Dosage Forms

Sequence	Event
1	Evening meal (~1000 kcal) at 14-hr pre dose
2	Overnight fast followed by blood glucose test on arrival at clinic
3	Dose in the morning following a light breakfast (~280 kcal)
4	Water allowed <i>ab libitum</i> but record the quantities ingested
5	Lunch (~1000 kcal) at 5-hr post dose
6	Afternoon snack (150 kcal) at 7.5-hr post dose
7	Evening meal (~1000 kcal) at 10-hr post dose

Source: From Ref. 48.

important to note that the volunteers should be ambulatory during the study period to simulate a normal day's activity (48).

SMALL INTESTINAL TRANSIT TIMES

In the small intestine, contact time with the absorptive epithelium is limited, and a small intestinal transit time (SITT) of 3.5 to 4.5 hours is typical in healthy volunteers. The Holy Grail of drug delivery would be to discover a mechanism that extended the period of contact with this area of the GI tract. Various approaches have been suggested, but a universal solution is not evident, and data demonstrating phenomena that extend GI residence are often subject to controversy. Attempting to examine the effects of altering the contact time of a drug with the small intestine by treatment with metoclopramide or propantheline bromide has been a classical stratagem ever since the first observations on the effects of these compounds on the absorption of the poorly soluble drug griseofulvin (49). Marathe and colleagues (50) examined the effects on metformin solutions labeled by addition of [^{99m}Tc]-DTPA. Metformin absorption, which is limited by poor permeability, began when the solutions entered the small intestine and started to decline when the material reached the colon. In those cases where propantheline was used to greatly increase the residence time in the small intestine, absorption appeared to be complete prior to arrival at the colon.

Infusion of fat into the ileum has been shown to cause a lengthening of the SITT—a phenomenon known as the *ileal brake* (51,52). However, the effect is generally modest (causing a delay of 30–60 minutes) and attempts to exploit this mechanism in drug delivery have had limited success. Dobson and colleagues studied the effect of coadministered oleic acid on the small intestinal transit of nondisintegrating tablets (53,54). They showed a delay in SITT in over half of all cases, and a doubling of SITT in some instances, but in the other cases SITT was either unaffected or even reduced. Lin and colleagues have also showed slowed GI transit in patients with chronic diarrhea by administration of emulsions containing 0, 1.6, and 3.2 g of oleic acid (55). Small intestinal transit in *normal subjects* was measured at 102 ± 11 minutes, while the transit times in the *patients* treated with the three emulsions were respectively 29 ± 3 , 57 ± 5 , and 83 ± 5 minutes.

MOTILITY AND STIRRING IN THE SMALL INTESTINE

Muscular contractions in the wall of the small intestine have to achieve two objectives: first, stirring of the contents to increase exposure to enzymes and to bring the lumenally digested products close to the wall and second, propulsion

of indigestible material toward the distal gut. To accomplish this, movements of the gut consist of a mixture of annular constricting activity (segmentation) together with peristaltic movements, which are of both long and short propagation types.

Many of the new generation of drugs have issues with regards to solubility or effective forward (lumen to systemic blood supply) flux, resulting in low bioavailability. Sufficient residence time and mixing is, therefore, needed for drugs to be solubilized, and the effects of fat on motility are of especial interest in the formulations of poorly water-soluble lipophilic compounds. Fat infusions into the proximal gut increase the rate of transit through the proximal small intestine but cause a delayed transfer of material though the ileocecal junction (56). In contrast, when fat appears in the distal gut, the upper gut propulsion is reduced by around 30% (57). Detection of fatty acids by the ileum causes a release of peptide YY (PYY), which appears to be able to directly act on the vagal efferent branches supplying the duodenum and jejunum (58). This suggests that there must be two pathways operating: an enterogastrone route supplemented by a local extended neural network (the so-called "brain in the gut") more properly termed the enteric nervous system (ENS) and extrinsic nerve action, specifically the vagus activated by PYY. The chain is quite complex and multistage. Data from Lin et al. showed that ileal luminal fat might work though a serotonergic receptor situated in the ileum with the slowing signal being carried successively by PYY, a β -adrenergic pathway, a serotonergic pathway, and an opioid pathway (59).

γ -Scintigraphy is not well suited to the study of real-time movement, although Kaus and colleagues applied the technique to measure the average transit rate through the jejunum and ileum of a Perspex capsule labeled with Tc-99m (60). Real-time imaging techniques with high spatial resolution, such as magnetic moment monitoring, allow nonradioactive methods to examine the pattern of movement of capsules through the GI tract (61). The technique involves the incorporation of a small amount of iron oxide into the formulation and detecting the tiny induced magnetic field against the earth's magnetic field. The GI transport of dosage forms using MMI reveals that movement of the formulation in the stomach as well as in the small and the large bowel is extremely discontinuous. Transit of solid dosage forms through the small intestine is characterized by consecutive phases of rest or slow propagation with highly variable duration and typically brief motility events with velocity spikes of up to more than 50 cm/sec (Fig. 5).

This observation is in agreement with the characteristics of intestinal propulsion of chyme, as movement of chyme is characterized by periods of slow transit that alternate with bursts of rapid flow (62). Therefore, discontinuity in transport can be regarded as a distinguishing feature of GI transit.

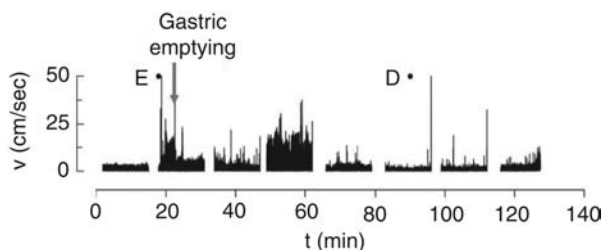


FIGURE 5 Velocity profile of an enteric-coated tablet form intake until disintegration. E indicates emptying from the stomach and D time of disintegration.

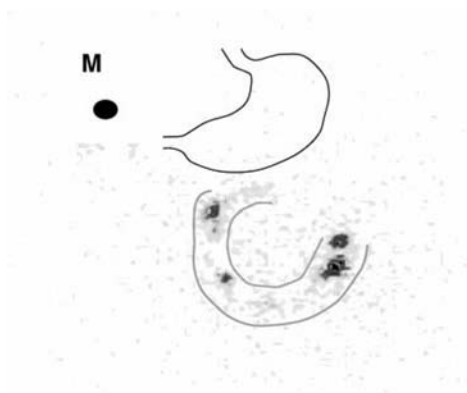


FIGURE 6 γ -Scintigraphic images of small intestinal transit of capsules showing periods of stasis during a 30-second acquisition. M = exterior marker.

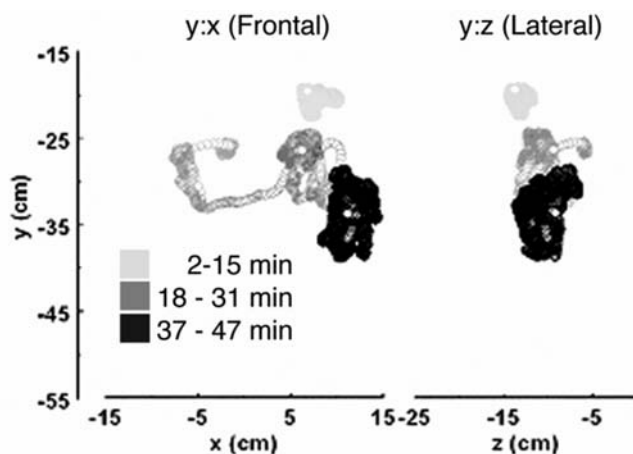


FIGURE 7 Magnetic moment images of an enteric-coated tablet containing a small amount of magnetized ferric oxide. Left-hand panel shows three sequences in a single volunteer viewed from the front. The right-hand panel shows the same sequences viewed from the top. *Source:* From Ref. 110.

In a γ -camera image, periods of stasis can also be observed as illustrated in Figure 6. The visualization of a tablet in real time is best illustrated using MMI as shown in Figure 7.

The passage of an enteric-coated tablet moving through the gut of a volunteer was monitored over three periods of time up to 47 minutes post administration. The greater rate transit through the upper gut is clearly seen in the middle period—18 to 31 minutes—when the unit travels through the duodenum. Differences in applied agitation forces on the formulation in four volunteers are evident in Figure 8. Comparing formulation movements during the time the unit is in the stomach and in the upper intestine, as shown in Figures 5 and 7, suggests that the period of contact with the mucosa is low in these regions compared to further along the gut.

As might be expected, the presence of nutrients in the gut alters motility—drinking glucose solutions or Intralipid[®] increases contraction of the gut

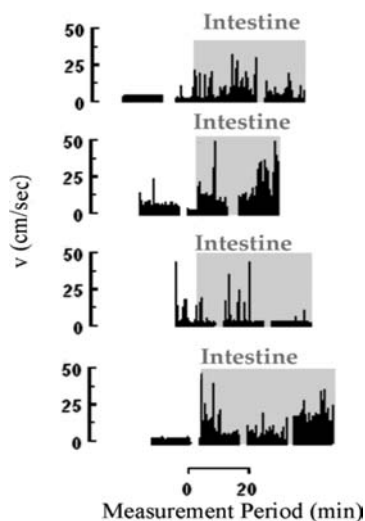


FIGURE 8 Differences in transit velocities in four subjects, before and after leaving the stomach.

significantly. Both increase contractions to the same extent, with the duration of the increase dependent on caloric activity (63). The same group, von Schonfeld and colleagues, had previously showed that increasing the viscosity of the gastric contents by administration of guar (5 g) delayed gastric emptying of the glucose load (300 kcal in 300 mL water) and produced a prolongation of the postprandial contractile activity (64). The effect was seen when the guar was given with a meal, but not with water, suggesting that the guar effect is due to a slowed delivery of calories from the stomach and perhaps from the intestinal lumen.

Exposure of the intestinal cells to high concentrations of the solubilizing excipient polyethylene glycol 2000 causes villus shortening, goblet cell capping, and destruction of the villus tip (65). The effects of smaller molecular weight-solubilizing excipients were more extreme and were not tolerated by the intestinal tissue. Contact with strong osmotically active agents would be expected to reverse water flux from the tissues and cause contractions. Basit and colleagues recently reported a study in which a 150 mL orange juice drink containing 10 g PEG 400 was given with an immediate release pellet formulation containing 150 mg ranitidine (66). The control was the juice without PEG 400 and the liquids were tagged with In-111 to allow measurement of transit. Mean small intestinal transit was decreased from 226 to 143 minutes and the absolute bioavailability of ranitidine decreased by a third.

At least under fasting conditions, the small intestine does not represent a tube that is homogeneously filled with water. In an imaging study using MRI, it was demonstrated that small intestinal water is distributed to form some "pockets" (typically 4 to 6) with a total mean volume of less than 100 mL (67). Measured water volumes and distribution are shown in Figure 9. Accordingly, during small intestinal transit nondisintegrating dosage forms are not necessarily permanently in contact with water. Furthermore, water that is swallowed and drug substances may follow different routes of absorption from the gut.

The proximal small intestine is capable of absorption of about 8 L water per day at a rate of about 50 mL/min via a cascade of apical and basolateral

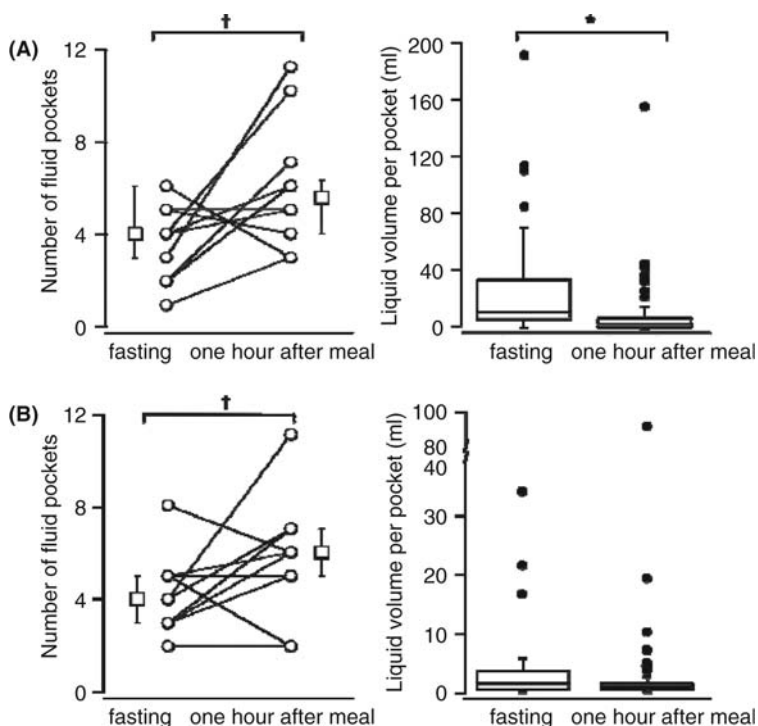


FIGURE 9 Number of fluid pockets (*left, circles*) and liquid volumes per pocket (*right*) in small intestine (**A**) and large intestine (**B**) under fasting conditions and one hour after ingestion of a meal. The boxes show the median with 25% and 75% quartiles. † $p < 0.05$, Chi square test, * $p < 0.001$, Mann-Whitney test. *Source:* From Ref. 67.

solute carriers located on the surface of enterocytes and via endothelial pores of mucosal capillaries. The key features include the sodium/glucose cotransporter SGLT1 and aquaporins, notably AQP1, AQP3, APQ8, and APQ10 (68–71). A surprisingly common observation in the resting small intestine is the presence of water pocket in the terminal ileum close to the ileocecal junction.

COLON: WATER CONTENT

For most formulations, colonic absorption represents the only real opportunity to increase the interval between dosing. Transit through the lower part of the gut is quoted at around 24 hours but in reality only the ascending colonic environment has sufficient free fluid to facilitate dissolution. The supplementation of diet by fiber increases the water content of the colon—undigested insoluble fiber carries about 2 mL water per gram of dry weight (72) but effectiveness of fiber in easing functional constipation appears to require an additional intake of 1.5 to 2 L of extra fluid a day (73). Soluble fibers have a higher capacity for retaining water, at least *in vitro*, swelling more than 20 times their dry weight (74).

The impact of this large amount of hydrogel on drug dispersion in the colon has not been investigated but remains a subject of considerable interest. In our MRI study on intestinal water contents and distribution, we determined a

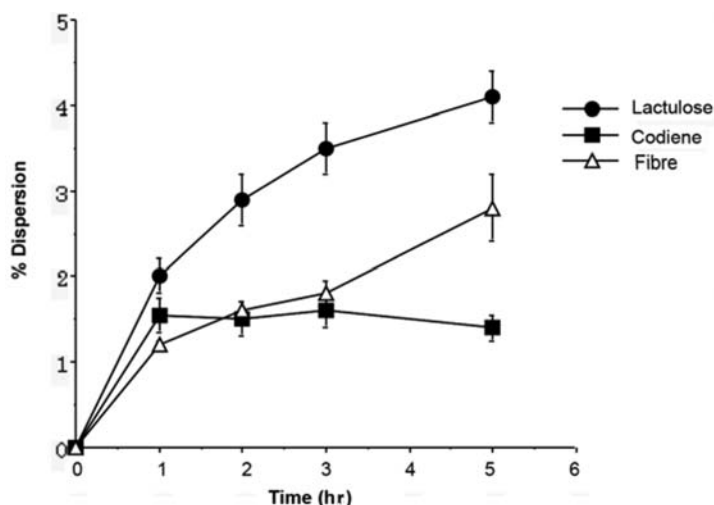


FIGURE 10 Dispersion of material following timed release in the colon using Pulsincap® devices.

total volume of about 15 mL of free water in the colon (67). This finding is corroborated by data obtained in a study performed by Professors Reppas and Dressman where they aspirated comparable water volumes from the region of the ascending colon via a colonic catheter. In the colon, water availability is low past the hepatic flexure, as the ascending colon is extremely efficient at water and electrolyte absorption with a reported absorption capacity of about 3 mL water per minute (75). Release at the ileocecal junction, before significant absorption of luminal water has occurred, appears to provide satisfactory dispersion in the right colon. There is evidence suggesting that net absorptive water flux in the colon, in both the basal and postprandial states, appears to be augmented by intraluminal glucose (76). Further, changing the water content of the human colon by coadministering 20 g lactulose for three days markedly increases dispersion and dissolution in the transverse colon, as shown for subjects dosed with quinine sulfate in a colon-targeted device in Figure 10.

Motility changes in the colon can also be brought about by bacterial overgrowth and there is a school of thought that believes that patients with irritable bowel syndrome show symptoms, which are similar to those of small intestinal bacterial colonization. It would be expected that the overgrowth would produce contraction and segmentation leading to stasis and pockets of gas in the bowel. Indeed, eradication of overgrowth with antibiotics appears to be associated with relief of symptoms in irritable bowel syndrome as judged by standard assessment criteria (77).

COLONIC GAS

In the cecum, the fermentation of any soluble fiber present produces short-chain fatty acids (SCFA) and gas (largely carbon dioxide, but with small amounts of hydrogen and methane if the redox conditions are appropriate). In vitro fermentation studies of fiber with a human fecal inoculate show that the amount of

gas produced correlates approximately with SCFA production and varies with the fiber type. In the studies described by Campbell and Fahey (78), pectin produced the most gas during extended fermentation (108 mL/g), whereas methylcellulose produced only 0.57 mL/g. Considerable inter and intrasubject variability in potential in vivo fermentation of pectin-containing vegetables has been noted (73), which may be due to the presence of other bacterial commensals. In fecal incubations from pigs fed probiotic bacteria (live lactobacilli), carbon dioxide production was reduced, although hydrogen sulfide production was increased (79). When *Lactobacillus plantarum* was dosed to patients with irritable bowel syndrome, flatulence decreased and less pain was reported in the test versus the placebo group (80).

The gas rises into the transverse colon and can form temporary pockets, which can restrict access of water to the formulation, particularly if the dosage form does not permit uptake of water through the surface. For this reason, distal release of drug can be hampered by poor wetting/spreading and the reduced surface area, leading to restricted absorption. In an unpublished study, MRI was used to investigate changes in colonic volume after ingestion of fermentable fiber. Twelve subjects received sachets of Fybogel[®] and the gas volumes measured at 5 pm before the fermentable fiber regimen and after four days of dosing. Gas volumes of ascending, transverse, and descending colon were measured by MRI, quantifying the gas by addition of slice volume in the images corresponding to air, using the histogram function in Photoshop[®]. The data show remarkably consistent volumes in the mean total volumes, with shifts as anticipated in ascending and transverse colon (Fig. 11).

Drugs that affect transit time would be expected to alter the normal flora and metabolic activity of the colonic lumen. Oufir et al. investigated the effects of treatment with cisapride and loperamide on fecal flora and SCFA production (81). By doubling the transit time with loperamide, the concentration of SCFAs were markedly increased, whereas by reducing the transit time with cisapride, pH was elevated and the concentration of SCFAs was significantly reduced.

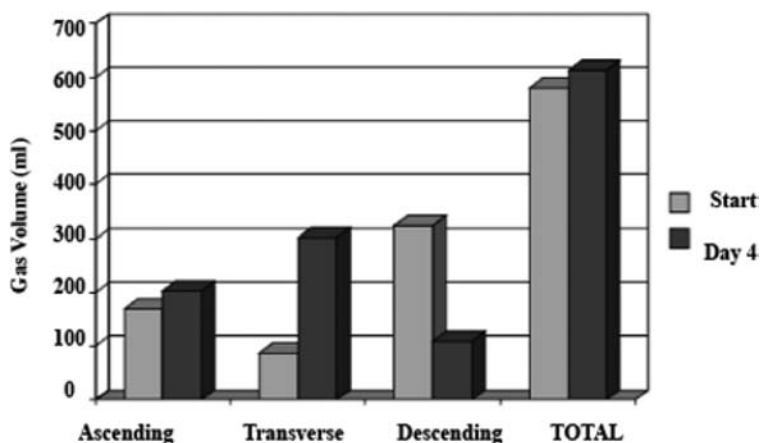


FIGURE 11 No change in mean colonic gas volumes after four days treatment with a soluble fiber supplement. Source: Wilson, unpublished data.

The finding that a gas, nitric oxide, was a neurotransmitter caused great excitement when it was discovered to be important in signaling and that chronic production was associated with direct tissue toxicity, inflammation and cancer. As has been stated, several gases are produced by fermentation in the gut, especially carbon dioxide. In some individuals, the redox potential is sufficient to produce hydrogen and even methane, which can be detected in the breath. Methane is detectable in a third to two-thirds of the population and can be seen after dosing patients with small bowel overgrowth after a dose of lactulose. The detection was associated with patients with constipation (82), which prompted Pimental's group to examine a role for methane in small intestinal motility (83). Instillation of small amounts of methane gas into the dogs with small intestinal fistulae and comparison with isolated guinea pig ileum led the workers to conclude that methane augments the slower intestinal motility. Moreover, patients with IBS, who were methane producers, were found to have a higher motility index than hydrogen-producing IBS subjects. Higher bowel activity is associated with constipation, which may explain the increased symptoms in this group.

DISTRIBUTION OF MATERIALS IN THE COLON

Our early scintigraphic studies, in which Tc-99m pellets and In-111-labeled nondisintegrating tablets were dosed together, suggested differential transit through the lower gut (9). This was confirmed in later studies in which small tablets and pellets labeled with In-111 and Tc-99m were dosed in colon-targeted dosage forms (84). The pellets appear to become trapped in the plaecal folds, whereas the solid units were propelled forward. This has been a consistent finding, which has great importance in terms of dosage form design to prolong release in the gut. Other workers using inert plastic flakes and granules have also investigated shape factors of nonnutrients on whole-gut transit time (85). The plastic flakes showed a more rapid transit than the granules, supporting the scintigraphic evidence.

The anatomy of the distal colon, with its thick muscular walls, suggests a predominantly propulsive activity. Studies with single administrations of pellets or Pulsincap[®] devices suggested that the distal part of the transverse colon area is difficult to treat, since this area and the descending colon function as a conduit. Steady state measurements confirm this assertion (86). Colon targeting is severely hampered by the observation that colonic transport is dominated by events of mass movement (87,88). Mass movements as well as the propulsion of the contents of the terminal ileum into the ascending colon are stimulated by food intake (67,89).

To estimate the probable duration of treatment with topical agents, we conducted studies in normal subjects and patients with left-sided colitis (i.e., disease predominantly affecting the descending colon). The subjects and patients were dosed daily with In-111-labeled Amberlite resin and imaged throughout the day. On the fourth, the division of activity in the colon was 67% in the proximal half and 33% in the distal half day for the control subjects, whereas for the patients with colitis the distribution was 90:10. These data emphasize the problem of treating left-sided colitis effectively during active periods of disease (86).

THE IMPORTANCE OF TIME OF DOSING

Time of dosing appears to be a further important factor in maximizing colonic contact, particularly in the ascending colon. Morning dosing without fasting is a common regimen in clinical trials, and patterns of motility under these conditions, at least in healthy volunteers, have been well established using scintigraphy. Following early morning dosing, a nondisintegrating unit clears the stomach in one to two hours and has a small intestinal transit time of 3.5 to 4.5 hours, although transit times as short as 2 hours or less have been noted in a few individuals. For most subjects dosed at 8 a.m., the unit will be expected to be at the ileocecal junction or to have entered the colon by around 1 p.m. Colonic transit through the proximal colon of intact objects such as nondisintegrating capsules is usually 5 to 7 hours, whereas transit of the dispersed particulate phase is longer, around 12 hours (90,91). For a nondisintegrating object dosed in the morning, the unit will have arrived at the hepatic flexure by 7 to 8 p.m. Thus, assuming the drug is absorbed in the colon, the typical time window for absorption is 6 to 10 hours following morning dosing with a monolith and 12 to 15 hours with particulates.

Studies using the Pulsincap system (92) were carried out with the objective of targeting the distal colon with a pulsed delivery of a transcellular probe (quinine) and [^{51}Cr]-EDTA, a paracellular probe. In these studies, subjects were dosed at 10 p.m. to ensure delivery to the descending colon by lunchtime the following day. The site of release was identified by incorporating [^{111}In]-labeled resin into the unit and imaging the subjects with a γ -camera. A total of 39 subjects were investigated. Fifteen hours after nocturnal administration, the majority of the delivery systems were situated in the proximal colon at their predicted release time and had not advanced further than a similar set of systems viewed only six hours after dosing. This relative stagnation appears to reflect the lack of propulsive stimuli caused by the intake of food, and the effect of sleep in reducing colonic electrical and contractile activity (93–96). Delayed nocturnal gastric emptying (97) and reduced propagation velocity of the intestinal MMC (98) may also have contributed, as supported by the finding that in two individuals the delivery system did not enter the colon until 12.5 and 13.5 hours after ingestion.

If a delayed release formulation is taken around 5 pm, it will have progressed through the ascending colon by the time the patient goes to bed. Quiescence of propulsive movements in the large bowel causes a relative stagnation, and units remain in the ascending colon overnight. Potentially, this can increase the time of contact to 11 to 13 hours even for a slowly dissolving matrix. On rising, the change in posture stimulates mass movements, felt by the subject as the urge to defecate, and contents move from the right to the left side of the colon.

From the studies conducted using γ -scintigraphy and MRI, it can be concluded that both temporal and dietary factors are important codeterminants of transit. In addition to modified release dosage forms, the maximum time window for absorption is an important determinant of bioavailability for some poorly soluble substances. Brocks and colleagues describe a significantly higher exposure of a leucotriene receptor (LTD4) antagonist (pranlukast) administered as a 300 mg oral dose 2000 to 2100 hours versus 0800 to 0900 hours in a randomized crossover design (99). The compound, which has a solubility of 0.9 $\mu\text{g/mL}$ in water at 25°C, was administered 30 minutes after a standard high-fat breakfast. The results are shown in Figure 12.

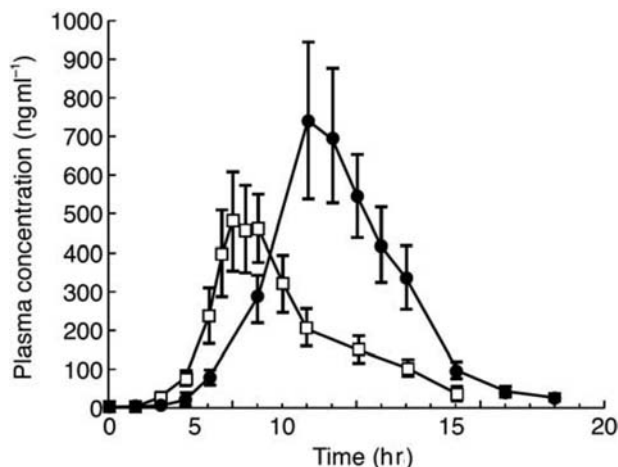


FIGURE 12 Mean plasma pranlukast concentrations after a 300 mg oral dose taken with a high fat breakfast in the morning (*open squares*) or at night (*closed circles*). *Source:* From Ref. 99.

Measured half-lives of the terminal phase of elimination were similar and there was an increase in geometric mean AUC (156%) and $C_{\max}$ (114%) relative to the morning dose. The authors attribute the increase in AUC to increased intestinal blood flow, coupled with a slower rate of emptying. The principal site of pranlukast absorption is thought to be the duodenal segment.

Moving away from the current practice of dosing once-a-day formulations in the mornings might allow a reduction in the dosing frequency and increased efficacy of colon-targeted drugs and for formulations used to prevent acute disease episodes at night and in the early morning.

EFFECTS OF AGE, GENDER, AND OTHER FACTORS

Physiological functions naturally change with advancing age. However, there has always been great debate about the magnitude of age, gender, and other nonmeal-related factors, including posture and exercise, on GI transit (100). It is now generally accepted that gastric emptying and colonic transit are prolonged in women compared with those in men (101). However, there is still some debate about the effects of gender on SITT. Bennink and colleagues concluded that SITT of a dual radionuclide-labeled test meal in healthy men and women are the same (102). Madsen's group has conducted studies on GI transit using a similar meal on various cohorts of healthy subjects utilizing γ -scintigraphy over a number of years. In a recent publication, the group concludes that age and gender do have an effect. Their measurements indicated that women have slower GI transit than men in all regions of the GI tract, particularly with regard to a slower mean colon transit in middle age. In contrast, aging was shown to accelerate the gastric emptying and intestinal transit significantly (103). A recent study showed that postprandial proximal gastric relaxation in women was prolonged, which is consistent with delayed gastric emptying (104). The differences in GI transit between the sexes have been attributed to the actions of female sex hormones. A study by Hutson and colleagues found that

premenopausal women, and postmenopausal women taking hormone replacement therapy (HRT), showed slower gastric emptying of solids than postmenopausal women not taking HRT (105). Furthermore, those postmenopausal women not taking HRT showed similar gastric emptying times to men. That being the case, one would expect that the fluctuations of female sex hormones during the menstrual cycle would also have an effect. Again, studies on this topic have yielded conflicting results: some studies have shown that GI transit is delayed during the luteal phase of the menstrual cycle (105,106), while others have found no effect (96,107,108).

Quigley's group in Cork, Ireland, have concluded that normal aging is associated with changes in motility but the pattern is varied and no clear clinical consequence can be identified (109). More important in their view are the pathophysiological influences including depression (and treatment with anticholinergics and opiates), hypothyroidism, and chronic renal failure.

CONCLUDING REMARKS

The relationship between GI transit and drug absorption is well established and investigative tools such as γ -scintigraphy, MRI, and MMI have greatly contributed to our understanding. In recent years, the Biopharmaceutics Classification Scheme has helped the industry contain costs in clinical development, and by appropriate choice of in vitro methods, we have a reasonable level of assurance that, for certain classes of compounds, we can reasonably predict performance on the basis of laboratory tests. There is no doubt that the issues of dissolution, absorption, and transit are the key variables for simple tablet, pellet, and capsule formulations. For more sophisticated formulations, particularly delayed release preparations, the situation is probably too complex to allow adoption of standard compendial dissolution tests irrespective of the choice of dissolution media. Our ability to progress in this area is dependent on arriving at a better understanding of the stirring and viscosity characteristics of the lower small intestine and large bowel. This will require more investment in the development of investigative methods and multimodal imaging to ascertain the true conditions experienced by a formulation in the unprepared human bowel.

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INTRODUCTION

Most drugs are taken orally. For those intended to act systemically, this route is not always the most efficient due to the numerous anatomic and physiologic barriers that drugs can encounter from the time of ingestion until the time of entry into the general circulation. As a consequence, before the drug enters the circulation and elicits its effects in the target tissue(s), significant loss of the original dose can occur as drug passes, sequentially, through the gastrointestinal (GI) tract, the liver, and the cardiopulmonary system. For some drugs, these barriers can even preclude their use as oral agents. Isoproterenol, dihydroergotamine, lidocaine, nitroglycerin, fentanyl, and naloxone are examples of drugs that suffer from a high *first-pass effect*, which refers to the loss of drug as the dose passes, for the first time, through organs of elimination during transit from the site of administration to the systemic circulation (1). Processes known to cause significant loss of active drug during first pass include incomplete release from the dosage form, degradation in the GI lumen, poor permeation through the GI wall, active export into the GI lumen, biliary excretion, and metabolism. Of these processes, only metabolism can take place in all of the aforementioned organs.

Enzymatic modification of drugs via phase I reactions (e.g., oxidation, reduction, and hydrolysis) and/or phase II reactions (e.g., sulfation, glucuronidation, and acetylation) generally produces inactive metabolites with increased polarity and water solubility to enhance excretion. For several drugs, the extent of conversion to inactive metabolites can be large enough such that circulating concentrations of active drug are reduced significantly, which in turn can cause a significant decrease in pharmacologic activity and, ultimately, a reduced clinical response. Drugs with a narrow therapeutic window that undergo extensive first-pass metabolism are, particularly, vulnerable to a reduced clinical response. Moreover, the extent of first-pass metabolism can vary substantially between individuals, further hampering the optimization of oral drug therapy.

Of the first-pass organs of drug elimination, the liver is the most often implicated, in large part, because it expresses the highest specific contents of drug-metabolizing enzymes. Next to the liver, the small intestinal mucosa is undoubtedly the most important extrahepatic site of drug metabolism (2). Accordingly, for drugs taken orally, the opportunity exists for sequential first-pass metabolism by the intestine and liver. While the role of the relevant enzymes in the liver has been established for some time, relatively less is known about the complement of enzymes in the small intestine. Nevertheless, much progress has been made in the last two decades regarding the identification and characterization of different subfamilies and individual isoforms. In parallel, evidence has amassed that the small intestine can contribute significantly to the overall first-pass metabolism of drugs, the extent of which can have clinical ramifications.

CLINICAL IMPLICATIONS OF INTESTINAL FIRST-PASS METABOLISM

Many commonly prescribed drugs undergo extensive first-pass metabolism upon oral administration (Table 1). For those listed in Table 1, at least 45% of the original dose is lost, on average, before entering the systemic circulation. That is, all have a low average *oral bioavailability* (F_{oral}), which refers to the fraction of the oral dose that reaches the systemic circulation in the unchanged form. Since metabolism is frequently the major source of first-pass drug elimination, F_{oral} is often used to assess the extent of first-pass metabolism. F_{oral} can be calculated from the ratio of the area under the blood or plasma concentration–time curve following oral administration (AUC_{oral}) to that following intravenous administration (AUC_{iv}) after correcting for dose (equation 1):

$$F_{\text{oral}} = \frac{\text{AUC}_{\text{oral}}}{\text{AUC}_{\text{iv}}} \times \frac{\text{Dose}_{\text{iv}}}{\text{Dose}_{\text{oral}}} \quad (1)$$

TABLE 1 Selected Drugs with Low and Variable Oral Bioavailability Believed to Be Due in Part to Intestinal First-pass Metabolism

Drug	Intestinal enzyme(s)	Oral bioavailability (%) (Average $\pm$ SD)
Alfentanil	CYP3A (3)	43 $\pm$ 19
Amiodarone	CYP3A	46 $\pm$ 22
Atorvastatin	CYP3A	12
Buspirone	CYP3A	3.9 $\pm$ 4.3
Cyclosporine	CYP3A	28 $\pm$ 18
Diclofenac	CYP2C9	54 $\pm$ 2
Dihydroergotamine	CYP3A (4)	0.5 $\pm$ 0.1 (5)
Diltiazem	CYP3A	38 $\pm$ 11
Erythromycin	CYP3A	35 $\pm$ 25
Ethinyl estradiol	CYP3A, SULT1E1, UGT1A1 (6–9)	42 (8)
Felodipine	CYP3A	15 $\pm$ 8
Fluvastatin	CYP2C9	
Irinotecan	CYP3A, CES2 (10–12)	8 (12)
Isoproterenol	SULT1A3 (6,7)	28 (6)
Lidocaine	CYP3A	35 $\pm$ 11
Losartan	CYP2C9, CYP3A	36 $\pm$ 16
Lovastatin	CYP3A	$\leq$ 5
Midazolam	CYP3A	44 $\pm$ 17
Nicardipine	CYP3A	18 $\pm$ 11
Nifedipine	CYP3A	50 $\pm$ 13
Omeprazole	CYP2C19, CYP3A	53 $\pm$ 29
Oxybutinin	CYP3A	1.6–10.9
Raloxifene	UGT1A1, UGT1A8, UGT1A10 (13)	2
Saquinavir	CYP3A	4–13
Sirolimus	CYP3A	15
Tacrolimus	CYP3A	25 $\pm$ 10
Terbutaline	SULT1A3 (7)	14 $\pm$ 2
Triazolam	CYP3A	44
Verapamil	CYP3A, CYP2C9	22 $\pm$ 8

Enzyme(s) and bioavailability values are from Ref. (14) unless indicated otherwise.

Abbreviation: CYP, cytochrome P450; SULT, sulfotransferase; UGT, UDP-glucuronosyl transferase; CES2, carboxylesterases 2.

When drug-metabolizing organs are arranged sequentially, such as the small intestine and liver, F_{oral} may be viewed as the product of the fractions of the dose that escape first-pass metabolism by each organ (equation 2):

$$F_{\text{oral}} = F_{\text{abs}} \times F_I \times F_L \quad (2)$$

where F_{abs} is the fraction of an oral dose absorbed intact through the apical membrane of the enterocyte, and F_I and F_L are the fractions of the absorbed dose that escape metabolism by the intestine and liver, respectively. In terms of extraction ratios (the fractions that do not escape first-pass metabolism), equation 2 can be rewritten as follows (equation 3):

$$F_{\text{oral}} = F_{\text{abs}} \times (1 - E_I) \times (1 - E_L) \quad (3)$$

where E_I and E_L are the extraction ratios associated with the intestine and liver, respectively. Equation 2 illustrates the impact of a second presystemic site of metabolism on F_{oral} . For example, if the entire dose is absorbed into the enterocytes intact ($F_{\text{abs}} = 1$), has an F_I of 60%, and has an F_L of 40%, then an F_{oral} of 24% is predicted. If only first-pass metabolism by the liver is considered ($1 \times 1 \times 40\%$), then an F_{oral} of 40% is predicted. Thus, omission of the intestinal component would result in an overestimation of F_{oral} , which could potentially lead to suboptimal dosing and ineffective concentrations at the site(s) of action. For drugs that have a wide therapeutic window, this situation can be rectified simply by increasing the dose. However, for drugs that have a narrow therapeutic window, optimization of oral dosing regimens becomes more challenging. Moreover, factors that significantly alter metabolism, including other xenobiotics that induce or inhibit drug-metabolizing enzymes, thus altering F_I and/or F_L , present further challenges to optimal oral drug therapy.

Equation 2 also illustrates the potential impact of a second presystemic site of metabolism on the interindividual variation in F_{oral} . For example, if a drug has an F_I that varies from 30% to 60% (twofold range), an F_L that varies from 20% to 80% (fourfold range), and the extraction efficiencies of the gut and liver vary independently, then F_{oral} will vary from 6% to 48% (eightfold range), resulting in a 100% increase in the variation in F_{oral} (if only F_L were considered initially). Indeed, data collected from 143 pharmacokinetic studies showed a significant inverse correlation between F_{oral} and the interindividual variation in F_{oral} , as measured by the coefficient of variation in F_{oral} (15). This relationship indicated that the greater the extent of first-pass elimination, the greater the variation in F_{oral} . Accordingly, knowledge of the degree and variation in the expression of the major drug-metabolizing enzymes in the human intestine is essential, as these enzymes can represent a key determinant of not only the extent of first-pass metabolism but also the interindividual variation in F_{oral} and the probability and magnitude of drug-xenobiotic interactions (16).

DRUG-METABOLIZING ENZYMES IN THE INTESTINAL WALL

The human intestinal wall expresses several of the same drug-metabolizing enzymes as the liver, including both phase I and phase II enzymes (Table 2), and are located predominately in the enterocytes. As with enzymes in the hepatocyte,

TABLE 2 Drug Metabolizing Enzymes in the Human Small Intestine That Are Known to Be Expressed at the Protein Level and to Have Catalytic Activity

Enzyme	Location in subcellular fraction
Phase I	
Cytochromes P450 (CYPs)	Microsomes
Carboxylesterases (CESs)	Microsomes, cytosol
Epoxide hydrolases (EHs)	Microsomes, cytosol
Flavin monooxygenases (FMOs)	Microsomes
Phase II	
Sulfotransferases (SULTs)	Cytosol
UDP-glucuronosyl transferases (UGTs)	Microsomes
N-acetyltransferases (NATs)	Cytosol
Glutathione S-transferases (GSTs)	Cytosol

enzymes in the enterocyte generally reside in either the microsomal or cytosolic fraction (Table 2). Compared to enzymes in the liver, research on the expression and catalytic properties of the complement of enzymes in the GI tract has lagged, largely due to a limited supply of high-quality tissue as well as the lack of sensitive methods to detect the low expression levels/catalytic activity relative to the liver. Over the past decade, however, a variety of human intestine-derived tissue preparations have become available, including subcellular fractions (microsomes, cytosol), precision-cut tissue slices, shed enterocytes, Ussing chamber preparations, and intestinal cell lines (17,18). Thus, through the application of the same molecular biologic techniques as for hepatic enzymes, coupled with the ongoing identification of selective catalytic probe substrates and inhibitors, and improved methods of detection, a rigorous characterization of the various intestinal enzymes has become more feasible.

Phase I Enzymes

Cytochromes P450

The cytochromes P450 (CYPs), the most prominent of the phase I enzymes, constitute a superfamily of heme-thiolate monooxygenases that catalyze the biotransformation of both endo- and xenobiotics, the latter including a myriad of widely prescribed drugs. Common reactions include hydroxylation, N- and O-dealkylation, and epoxidation. Individual CYP enzymes are classified according to amino acid sequence similarities and are designated by a family number, a subfamily letter, and a number for a member within the subfamily. In general, members of the same family have at least 40% amino acid sequence identity, and members of the same subfamily have at least 55% amino acid sequence identity and are located within the same cluster on a chromosome. For example, CYP2C9 and CYP2D6 belong to the same family (CYP2) but to different subfamilies (CYP2C and CYP2D, respectively). In humans, approximately 80% of oxidative metabolism and almost 50% of the overall elimination of commonly used drugs can be attributed to one or more of the various CYP enzymes that belong to three families (CYP1, 2, and 3) (19).

The existence of CYP protein and associated monooxygenase activity (7-ethoxycoumarin O-deethylation) in the human small intestine was first reported in 1979 by Hoensch et al. (20), who determined total CYP content in a small

number of surgical specimens. Average ($\pm$ SD) content, as measured by carbon monoxide difference spectra, declined from proximal to distal regions and ranged from 93 ± 19 to 35 ± 4 pmol/mg microsomal protein; 7-ethoxycoumarin O-deethylase activity paralleled this pattern. Similar values were reported later by other investigators (21,22). Thus, on a per milligram microsomal protein basis, total CYP content in the average adult small intestine ranges from approximately 10% to 30% of that in the average human liver. Almost a decade following the report by Hoensch et al. (20), a series of pivotal *in vitro* and *in vivo* studies by Watkins et al. identified CYP3A as the major CYP subfamily expressed in human enterocytes.

CYP3A

In vitro studies Utilizing mucosae isolated from jejunal sections from four surgical patients, Watkins et al. identified a CYP enzyme and associated mRNA that were recognized selectively by an anti-CYP3A1 murine monoclonal antibody (which detected all human CYP3A forms) and CYP3A4 (termed HLp by the investigators) cDNA, respectively (23). Using purified CYP3A4 as the reference standard, average ($\pm$ SD) CYP3A4 protein content in microsomes prepared from the specimens was comparable to that for liver microsomes prepared from four separate organ donors/surgical patients (70 ± 20 vs. 65 ± 20 pmol/mg). Moreover, the average CYP3A-catalyzed rate of erythromycin N-demethylation in jejunal microsomes was comparable to that for liver microsomes and was inhibited by anti-CYP3A1. Three years later, de Waziers et al. (24), by immunoblot analysis, quantified the levels of various CYP isoforms/subfamilies in microsomes prepared from the following human extrahepatic tissues: esophagus, stomach, duodenum, jejunum, ileum, colon, and kidney. These investigators reported that, next to the liver, the duodenum was the highest with respect to immunoreactive CYP3A protein, followed by the jejunum and then ileum. Average duodenal, jejunal, and ileal CYP3A content represented approximately 50%, 30%, and 10%, respectively, of average hepatic CYP3A content. Corresponding values for the remaining extrahepatic organs were less than 5%. Consistent with the report by Watkins et al., CYP3A was the dominant CYP expressed in all three regions of the small intestine. More recently, a comprehensive analysis of microsomes prepared from the duodenal/proximal jejunal portion of 31 unrelated human donor small intestines demonstrated CYP3A as the major "piece" of the intestinal CYP "pie," representing approximately 80% of total immunoquantified CYP protein (25) (Fig. 1). Subsequent to the earlier *in vitro* studies, the significance of intestinal CYP3A to first-pass drug metabolism *in vivo* was demonstrated.

In vivo studies The widely used immunosuppressive agent, cyclosporine, is notorious for having a large interindividual variation in F_{oral} , which has been reported to range from 5% to 89% for the conventional formulation (Sandimmune) (26) and from 21% to 73% for the microemulsion formulation (Neoral) (27–30). This property, coupled with a narrow therapeutic window, can lead to an under- or overdosing of the patient, which in turn can lead to graft rejection or toxicity. The low and unpredictable F_{oral} of cyclosporine was believed initially to result from erratic absorption through the intestinal lumen coupled with variable hepatic first-pass metabolism (i.e., a low and variable F_{abs} and F_L). However, Kolars et al. (31), after instilling cyclosporine into the duodenum of two patients

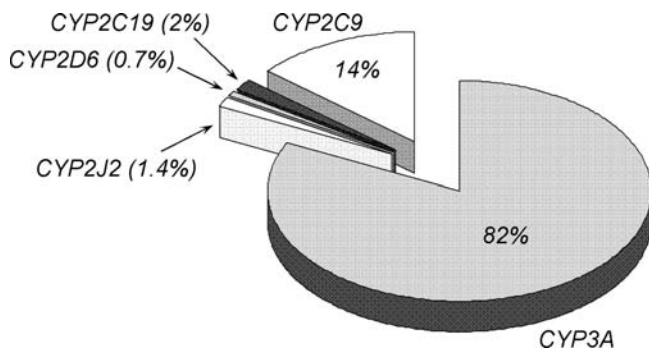


FIGURE 1 The average human proximal small intestinal cytochrome P450 “pie.” The percent contributions of individual enzymes are on the basis of average total immunoquantified CYP content (61 pmol/mg). *Source:* Adapted from Ref. 25.

during the anhepatic phase of their liver transplant operations, measured appreciable concentrations of two CYP3A-mediated primary metabolites in hepatic portal and systemic blood. The investigators concluded that the extra-hepatic site of metabolism was the gut because organs other than the gut (i.e., kidney and lung) express low levels of CYP3A, and portal metabolite concentrations exceeded systemic concentrations at the end of the anhepatic phase. These observations provided direct evidence that the small intestine can contribute significantly to the first-pass metabolism of a CYP3A substrate. A subsequent pharmacokinetic analysis of cyclosporine AUC after oral and intravenous administration suggested that the intestine, rather than the liver, was largely responsible for the first-pass elimination of cyclosporine (32). However, cyclosporine is now known to be a substrate for the efflux transporter P-glycoprotein (P-gp), which is expressed on the apical membranes of enterocytes and other cell types. As such, this indirect approach would not distinguish between intestinal CYP3A-mediated metabolism and P-gp-mediated efflux.

The sedative-hypnotic agent and CYP3A substrate midazolam has been shown not to be a substrate for P-gp (33), and thus should serve as a “clean” *in vivo* CYP3A probe. Using a study design similar to that for the cyclosporine study, the disposition of midazolam and its primary metabolite, 1'-hydroxymidazolam, was examined in a larger group of anhepatic transplant recipients following either intravenous ($n = 5$) or intraduodenal ($n = 5$) administration (34). Blood was collected simultaneously from the hepatic portal vein and a peripheral artery during the approximately one-hour anhepatic phase. Using the difference between the arterial and hepatic portal venous midazolam AUCs (intravenous) or between the hepatic portal venous and arterial midazolam and 1'-hydroxymidazolam AUCs (intraduodenal), an average ($\pm$ SD) extraction fraction of $8\% \pm 12\%$ and $43\% \pm 18\%$ was calculated for subjects who received midazolam by the intravenous and intraduodenal route, respectively. The low and variable extraction fraction following intravenous administration indicated that the intestine contributed somewhat to the systemic metabolism of midazolam. Importantly, the fivefold greater value following intraduodenal administration was identical to the E_I estimated in healthy volunteers ($43\% \pm 24\%$) (35). Moreover, these values were essentially identical to the E_L estimated

in the healthy volunteer study ($44\% \pm 14\%$) (35). These data strongly indicated that the small intestine is a major determinant of the overall extent of the first-pass metabolism of midazolam and can rival the liver.

By the indirect approach, enteric CYP3A also has been shown to contribute significantly to the first-pass metabolism of the calcium channel blockers nifedipine (36) and verapamil (37). The comparable contributions of the intestine and liver (mean E_I and E_L of 49% and 48%, respectively) to the overall first-pass elimination of verapamil was confirmed subsequently using a method involving a multilumen intestinal perfusion technique and stable isotope-labeled drug (38). This method demonstrated the importance of the intestine not only to the first-pass metabolism of verapamil but also to the secretion of verapamil metabolites, some of which are substrates for P-gp and possibly MRP2 (multi-drug resistance associated protein 2), another efflux transporter known to be expressed on the apical membranes of enterocytes. Indeed, intestinal secretion was shown to be as important as biliary excretion for the elimination of the metabolites.

For all of the aforementioned drugs, significant intestinal first-pass metabolism occurred despite evidence that total CYP3A content of the entire gut mucosa is only approximately 1% of total hepatic CYP3A content (70 vs. 5490 nmol) (21,39). Of apparently more importance than total enzyme mass is the comparable intracellular enzyme concentration (enterocyte vs. hepatocyte) and the obligatory nature of drug passage through the enterocyte (if transcellular absorption is operative). Thus, a more appropriate comparison might be of microsomal intrinsic activities. Indeed, mean CYP3A-mediated rates of erythromycin N-demethylation (23), tacrolimus O-demethylation (40), midazolam 1'-hydroxylation (21), and testosterone 6 β -hydroxylation (41) in small intestinal (duodenal/jejunal) microsomes were 45% to 120% of corresponding metabolic rates in hepatic microsomes. On the basis of these data, mean intestinal mucosal intrinsic clearances may be within two- to threefold of corresponding hepatic intrinsic clearances. Whether a similarity in vivo for a given drug will occur is more difficult to predict, as total oral dose, enzyme saturability, and absorption rate become relevant. Should the dose be large enough and the K_m of the drug for the enzyme active site be low enough, it is possible that the majority of absorbed drug could escape intestinal first-pass metabolism. Some of the HIV protease inhibitors (e.g., indinavir, saquinavir, and zidovudine) may represent such a case.

CYP3A4 vs. CYP3A5 As in the liver, CYP3A4 protein appears to be expressed constitutively in the small intestine of all individuals, whereas the closely related CYP3A5 is expressed polymorphically. For example, immunoreactive CYP3A5 protein was detected readily at a frequency of 20% to 30% in intestinal tissue obtained from adult Caucasians (21,42,43). Moreover, if detected, the enzyme was expressed along the length of the small intestinal tract (21). As has been shown for the liver (44), the frequency of CYP3A5 expression in the small intestine varies among different racial/ethnic groups (44). With the advent of specific, commercially available antibodies and suitable reference standards for immunoblot analysis, CYP3A5 has been shown to constitute from 3% to 80% of total intestinal CYP3A (CYP3A4 + CYP3A5) protein content (25,45). Accordingly, like hepatic CYP3A5 (44,46), enteric CYP3A5 may have a significant role in the first-pass metabolism of drugs in some individuals. Identification of a selective in vivo CYP3A5 probe substrate is needed to test this hypothesis.

Significant expression of CYP3A4 in the GI tract appears to be restricted to the small intestine. In mucosa of the stomach and colon, both CYP3A5 mRNA and protein were more prominent than corresponding CYP3A4 measures (47,48). Consistent with these observations, in two full-length human donor small intestines that were CYP3A5 positive, the ratio of CYP3A5 to CYP3A4 immunoreactive protein decreased from duodenum to jejunum, then increased in distal ileum to values comparable to or greater than those observed for the duodenum (49). Finally, Gervot et al. (50) detected CYP3A5 protein, but not CYP3A4 protein, in colonic mucosa from 40 unrelated and uninduced tissue donors. The authors suggested that any CYP3A4 in colonic tissue is likely to be a consequence of prior treatment of the donor with an enzyme inducer.

Localization of CYP3A enzymes CYP3A4 protein expression along the length of the small intestine is not uniform. Enzyme content is generally highest from duodenum to middle jejunum, then declines progressively to distal ileum (21,22,24). In microsomes prepared from mucosal scrapings obtained from 20 donor small intestines, median CYP3A4 content decreased from 31 to 23 to 17 pmol/mg protein in duodenum, jejunum, and ileum, respectively (21). CYP3A-catalyzed midazolam 1'-hydroxylation activity paralleled this pattern (21). Likewise, erythromycin N-demethylase activity decreased from proximal to distal regions (22). These data suggest that the extent of CYP3A-mediated first-pass metabolism may depend, in part, on the site of absorption.

CYP3A4 expression from the crypt to the tip of the small intestinal villus is also not uniform. By immunohistochemical analysis, CYP3A4 protein was not detected in the crypt cells or goblet cells but was readily detected in enterocytes, with the most intense staining evident in the mature enterocytes lining the villous tips (49,51). By *in situ* hybridization, a similar pattern was reported for CYP3A4 mRNA (48). Within the enterocyte, CYP3A4 protein was located predominately at the apex of the cell, adjacent to the microvillous border (48). The strategic location of CYP3A4 in mature enterocytes further highlights the small intestine as uniquely suited for the task of first-pass drug metabolism.

Agents and conditions that modify CYP3A activity Localization of CYP3A within only the mature enterocytes of the small intestinal mucosa is consistent with a wider pattern of differentiation of cell function as cells formed within the crypts migrate toward the villus tip and are eventually shed. Total CYP3A content, even within a defined region of the small intestine, varies considerably. CYP3A protein content measured in duodenal pinch biopsies obtained from CYP3A inducer/inhibitor-free healthy volunteers has been reported to vary approximately 10-fold (42,43). Even greater variability (>30-fold) was reported for CYP3A protein content and catalytic activity in duodenal, jejunal, and ileal mucosal scrapings obtained from 20 organ donors (21). Although some of the extreme variability in the latter study could have been due to events preceding organ procurement (e.g., reduced nutritional intake, antibiotic administration, brain death, and ischemia), these observations suggest that CYP3A is remarkably sensitive to a variety of modifying factors or conditions that can alter enzyme expression.

Dietary factors. One of the most extensively studied dietary substances in terms of CYP3A-mediated drug metabolism is grapefruit juice, which, when consumed in usual volumes, has been shown to elevate systemic concentrations of a variety of drugs by inhibiting intestinal, but not hepatic, CYP3A-mediated first-pass metabolism (52–55). The lack of an effect on hepatic CYP3A has been attributed to dilution of the causative ingredients in portal blood to concentrations below their effective inhibitory concentrations (K_i or IC_{50}) and/or to avid binding of the causative ingredients to plasma and/or cellular proteins in portal blood (56,57). The magnitude of the grapefruit juice effect can be large enough to cause untoward effects, such as severe muscle pain with some HMG-CoA reductase inhibitors (“statins”) (58,59) and hypotension/dizziness with some calcium channel antagonists (60). Accordingly, the labeling of several drug products contains precautionary statements regarding the concomitant intake of grapefruit juice. Using a “furanocoumarin-free” grapefruit juice suitable for human consumption and the CYP3A probe substrate felodipine, furanocoumarins were demonstrated unequivocally as major causative ingredients, several of which are potent reversible and mechanism-based inhibitors of enteric CYP3A catalytic activity (54,61). In addition, the pioneering study by Lown et al. (62) showed that grapefruit juice significantly reduced average enteric CYP3A4 immunoreactive protein (measured in duodenal pinch biopsies) by 60% in 10 healthy volunteers; the lack of a decrease in corresponding mRNA suggested a posttranscriptional mechanism. In vitro studies involving CYP3A4-expressing Caco-2 cells confirmed that two candidate furanocoumarins (bergamottin and 6',7'-dihydroxybergamottin) reduced CYP3A4 protein by accelerating enzyme degradation without affecting enzyme synthesis (63). The list of drugs shown to interact with grapefruit and related citrus juices is extensive and is described in several comprehensive reviews (52–55).

Therapeutic agents. Therapeutic agents that have been shown to inhibit intestinal CYP3A *in vivo* include the azole antifungals ketoconazole (64) and fluconazole (65); the macrolide antibiotics erythromycin (66), troleandomycin (3), and clarithromycin (67,68); and the calcium channel antagonist diltiazem (69). Exposure of human subjects to the enzyme inducer rifampin (7–10 days) and to the popular herbal medicine St. John's wort (14 days) increased average duodenal CYP3A protein content by ≥ 4 - and 1.5-fold, respectively, relative to baseline (37,51,70). Moreover, a comparison of the effect of rifampin on the systemic and apparent oral clearance of the CYP3A probes midazolam (3,71), triazolam (72), verapamil (37), nifedipine (36), and alfentanil (3) suggested that the inducer increased enteric enzyme levels to an extent greater than hepatic levels. The greater effect of enzyme inhibitors and inducers on enteric CYP3A activity compared to hepatic CYP3A activity may be due to higher intracellular concentrations and greater receptor occupancy in the enterocyte that occurs during absorption of the modifying agent.

Pathophysiologic conditions. Although less is known about the effect of disease on enteric CYP3A relative to hepatic CYP3A, some human studies have shown that pathophysiologic conditions can markedly alter enteric CYP3A protein expression/catalytic activity. For example, Lang et al. (73) reported that adult patients with celiac disease had reduced levels of jejunal mucosal CYP3A protein as a consequence of widespread epithelial cell destruction. Treatment with a

gluten-free diet reversed this aberration. Similar observations have been reported for pediatric patients with celiac disease (74). In the pediatric patients, after gluten rechallenge, a further decrease in enteric CYP3A expression was observed. Chalasani et al. (75) compared the disposition of midazolam between cirrhotic patients, cirrhotic patients with transjugular intrahepatic portosystemic shunts (TIPS), and healthy volunteers. The significantly higher mean F_{oral} in the cirrhotic patients with TIPS compared with that in the cirrhotic patients and healthy volunteers (0.76 vs. 0.27 and 0.30, respectively) was largely due to the significantly higher F_I in the TIPS patients compared with that in the cirrhotic and healthy subjects (0.83 vs. 0.32 and 0.42, respectively). The markedly lower extent of midazolam first-pass metabolism in the TIPS patients was concluded to result from diminished enteric CYP3A activity.

Intestinal vs. hepatic CYP3A. In view of the many differing responses between enteric and hepatic CYP3A to various regulatory factors, including those aforementioned, it follows that intestinal and hepatic CYP3A appear to be regulated independently. Such noncoordinate regulation was first demonstrated by Lown et al. (42), who reported that neither duodenal CYP3A protein content nor catalytic activity correlated with hepatic CYP3A activity in 20 healthy subjects. Likewise, other investigators found no rank order correlation between intestinal and hepatic CYP3A protein content or midazolam 1'-hydroxylation activity in microsomes prepared from eight matched intestine-liver donor pairs (21). Finally, independent groups of investigators found no correlation between the F_I and F_L of midazolam in healthy volunteers (35,67). This noncoordinate regulation between intestinal and hepatic CYP3A indicates that a measure of one should not be used to predict the other. However, the possibility of overlapping mechanisms of constitutive and inducible CYP3A expression cannot be excluded.

CYP1A1. CYP1A1 is expressed predominantly in extrahepatic tissues, including the lungs (76-78), placenta (79,80), stomach, and small intestine (25,81-83). In two independent investigations in which duodenal biopsies were obtained from healthy volunteers, CYP1A1 mRNA was expressed constitutively in all specimens; as with other CYP isoforms, large interindividual variation was evident among the specimens, at least sixfold (82,84). CYP1A1 protein and/or catalytic [ethoxyresorufin O-deethylase, (EROD)] activity were undetectable or low. Following treatment with the CYP1A inducers omeprazole (82) or chargrilled meat (84), enteric CYP1A1 protein and catalytic activity became readily detectable. Similarly, median duodenal EROD activity was higher in smokers and omeprazole-treated patients compared with nonsmoking control subjects (2.1 vs. 1.1 vs. 0.5 pmol/min/mg homogenate protein) (83).

Characterization of a bank of microsomes prepared from the proximal region of 18 human donor small intestines showed measurable rates of ethoxyresorufin O-deethylation in one-third of the donors, with a median and range (23.7 and 1.4-124 pmol/min/mg, respectively) (49) comparable to those reported for CYP1A2-catalyzed EROD activity in human liver microsomes (39.4 and 10.1-224 pmol/min/mg, respectively) (85). Median CYP1A1 protein content for the three preparations in which immunoreactive CYP1A1 was detected readily (5.6 pmol/mg) (25) was 14% of the average CYP1A2 protein content reported for a large panel of human liver microsomes (41 pmol/mg)

(86). The differing protein contents between enteric CYP1A1 and hepatic CYP1A2 despite comparable EROD activities were attributed to CYP1A1 having a greater catalytic efficiency than CYP1A2 toward the O-deethylation of ethoxyresorufin, as evidenced by recombinant CYP1A1 having both a lower K_m and a higher V_{max} compared with recombinant CYP1A2 (87 nM and 7.6/min vs. 240 nM and 1.9/min) (87). A greater catalytic efficiency for CYP1A1 compared to CYP1A2 also has been demonstrated for ethoxycoumarin O-deethylation and benzo(a)pyrene hydroxylation (88). In contrast, the catalytic efficiency of CYP1A1 toward the CYP1A drug substrates caffeine (89), theophylline (90), phenacetin (91), and *R*-warfarin (92) has been shown to be much lower than CYP1A2. Consistent with these observations, to date, there are no examples reported in the literature describing enteric CYP1A1 as having a significant role in the first-pass metabolism of drugs.

CYP2C9. Although CYP2C mRNAs have been detected in a number of human extrahepatic tissues (e.g., kidney, testes, adrenal gland, prostate, brain, and duodenum), significant protein expression appears to be limited to the small intestinal tract (24,93). de Waziers et al. (24) first detected what was described as "CYP2C8-10" in small intestinal microsomes, which, like CYP3A, was expressed predominantly in the proximal region. Other investigators later confirmed the descending pattern of expression of a CYP2C enzyme along the length of the small intestine (22). However, in both studies, it was unclear which enzyme (CYP2C8, CYP2C9, or CYP2C19) was detected. On the basis of the relative amount of each CYP2C enzyme in human liver, the intestinal form identified was most likely CYP2C9. From an analysis of 31 duodenal/jejunal microsomal preparations, two proteins were detected that reacted with a CYP2C-selective anti-CYP2C19 antibody and that comigrated with recombinant CYP2C9 and CYP2C19 protein standards (25). CYP2C9 protein content varied ninefold among the different preparations, with a mean specific content (8.4 pmol/mg) that was nearly one-tenth of reported average hepatic microsomal specific content (73 pmol/mg protein) (94).

With respect to intestinal CYP2C9 catalytic activity, Prueksaritanont et al. (95) reported a >20-fold variation in tolbutamide methylhydroxylase activity (<0.5–9.8 pmol/min/mg) for five duodenal/jejunal microsomal preparations; average ($\pm$ SD) activity (5.1 ± 3.8 pmol/min/mg) was at least one-tenth of the hepatic counterpart. Other investigators subsequently reported a similarly large interindividual variation in CYP2C9-catalyzed diclofenac 4'-hydroxylase activity (7.3–129 pmol/min/mg) for 10 human jejunal microsomal preparations; median activity was 55 pmol/min/mg (41), which was roughly one-sixth of that reported for a panel of 16 human liver microsomal preparations (~ 320 pmol/min/mg) (96). Collectively, these *in vitro* data suggest that the small intestine would have minimal contribution to the first-pass metabolism of drugs. However, because of the wide range in both specific content and activity, enteric CYP2C9 could be important in some individuals for substrates with a low F_{oral} , for example, fluvastatin (97). In addition, the low expression/catalytic activity of CYP2C9 in the intestine relative to the liver does not preclude the potential importance of enteric CYP2C9 to the first-pass metabolism of substrates ingested in trace amounts, for example, pesticides (98,99).

CYP2C19. CYP2C19 immunoreactive protein content for the aforementioned 31 human duodenal/jejunal microsomal preparations ranged from <0.6 to 3.9

and averaged 1.0 pmol/mg (25), which was one-fifteenth of average hepatic microsomal content (14 pmol/mg) (94). Large interindividual variation in enteric CYP2C19 catalytic activity also has been reported. CYP2C19-catalyzed 5-mephenytoin 4'-hydroxylase activity varied from 0.8 to 13.1 pmol/min/mg in the same panel of human small intestinal microsomal preparations that were analyzed previously for CYP2C9 activity (41). Average enteric catalytic activity (5.2 pmol/min/mg) was approximately one-tenth of the average activity reported for a panel of 10 human liver microsomal preparations (~45 pmol/min/mg) (100). As with enteric CYP2C9, these data suggest a minimal role for enteric CYP2C19 in the first-pass metabolism of drugs. The scarcity of CYP2C19 drug substrates with a low F_{oral} supports this contention. Again, the low enteric CYP2C19 expression/activity relative to hepatic CYP2C19 does not preclude the potential importance of enteric CYP2C19 to the first-pass metabolism of substrates ingested in trace amounts, for example, pesticides and insect repellents (101,102).

CYP2D6. CYP2D6 expression in the human intestine was first reported in 1990 by de Waziers et al. (24). Like CYP3A4, CYP2D6 protein was most concentrated in the proximal region and was localized in the enterocytes. The enzyme was not detected in ileum or colon. Prueksaritanont et al. later confirmed the expression of CYP2D6 protein in microsomes prepared from the proximal portion of two (103) and five (95) human donor small intestines. Moreover, CYP2D6-catalyzed (+)-bufuralol 1'-hydroxylation activity was measurable in all preparations. From a comprehensive comparison involving 19 human jejunal and 31 human liver microsomal preparations, CYP2D6 immunoreactive protein was detected readily in 18 of the intestinal preparations, with a median specific content (0.9 pmol/mg) that was one-fifteenth of the median content measured in the liver preparations (12.8 pmol/mg) (104). Median catalytic activity, as assessed by the intrinsic clearance of metoprolol oxidation, was also much lower in jejunal compared to hepatic microsomes (0.7 vs. 19.7 $\mu\text{L}/\text{min}/\text{mg}$). Likewise, the predicted average in vivo E_I for metoprolol was negligible compared to the predicted average E_L (0.01 vs. 0.48). The authors concluded that, unless a CYP2D6 substrate has a long residence time in the intestinal mucosa or undergoes futile cycling via an efflux transporter, enteric CYP2D6 would be expected to contribute minimally to the first-pass metabolism of drugs. However, enteric CYP2D6 may become clinically relevant if it mediates the formation of a cytotoxic metabolite that could cause mucosal damage (104).

CYP2J2. CYP2J2 is a relatively newly identified human CYP that is expressed predominately in extrahepatic tissues (105). Although most abundant in the heart, CYP2J2 is also expressed at appreciable levels (both mRNA and immunoreactive protein) in the GI tract (106). Immunoreactive CYP2J2 protein has been detected in microsomes prepared from the human esophagus, stomach, small intestine, and colon. Unlike other small intestinal CYPs, CYP2J2 expression was qualitatively highest in the esophagus and slightly lower but relatively uniform throughout the remainder of the GI tract (106). Moreover, there was little interindividual variation in CYP2J2 expression in jejunal microsomes. Although the role of CYP2J2 in drug metabolism remains largely unknown, in vitro studies have suggested that intestinal CYP2J2 contributes to the first-pass metabolism of the nonsedating antihistamines astemizole and ebastine.

Using human intestinal and liver microsomes, Matsumoto et al. (107) showed O-demethylation as the primary metabolic pathway for astemizole, with the average ($\pm$ SD) rate in enteric microsomes being approximately one-third of that in liver microsomes (170 ± 57 vs. 480 ± 88 pmol/min/mg). With recombinant CYP2J2 as the reference standard, immunoreactive CYP2J2 protein in microsomes prepared from five human small intestines averaged $2.1 (\pm 0.6)$ pmol/mg, consistent with that measured in a larger number of small intestinal microsomal preparations (1.0 ± 0.1 pmol/mg, $n = 31$) (25). These observations are comparable with average CYP2J2 content measured in liver microsomes from 20 Japanese and 29 Caucasian donors (2.0 ± 1.5 and 1.2 ± 2.1 pmol/mg, respectively) (108). A role for intestinal CYP2J2 in the O-demethylation of astemizole was supported further by the excellent correlation between CYP2J2 protein content and O-demethylastemizole formation rate in intestinal microsomes ($r = 0.90$, $p < 0.05$), as well as the strong inhibition of O-demethylastemizole formation by the CYP2J2 substrates ebastine and arachidonic acid. Using similar strategies, along with an inhibitory anti-CYP2J2 antibody, Hashizume et al. (109) demonstrated CYP2J2 as the major ebastine hydroxylase in human intestinal microsomes. Whether intestinal CYP2J2 contributes to the first-pass metabolism of astemizole, ebastine, and other drugs in vivo awaits further investigation.

CYP4F. CYP4F enzymes catalyze the biotransformation of several endogenous compounds, including arachidonic acid and its derivatives, such as leukotrienes, prostaglandins, lipoxins, and hydroxyeicosatetraenoic acids (110). Accordingly, the CYP4Fs are important regulators of vascular tone and inflammation, as well as other physiologic functions. In addition to their role in the biotransformation of endogenous compounds, the CYP4Fs have been reported to metabolize some drugs. For example, CYP4F12 was shown to be expressed, by reverse transcription polymerase chain reaction, in human liver and small intestine (111). Yeast-expressing CYP4F12 was capable of catalyzing the hydroxylation of ebastine, suggesting that CYP4F12 in the small intestine (and liver) may have a role in the first-pass metabolism of this drug. However, as reported subsequently by the same investigators, intestinal CYP2J2 was shown to be the predominate enzyme involved in this pathway (109). Most recently, Wang et al. identified CYP4Fs as the major enzymes in human proximal small intestinal microsomes that catalyze the initial O-demethylation of the antiparasitic agent pafuramidine (112). However, the much lower average intrinsic clearance of this reaction (0.3 ml/min/mg; $n = 9$) relative to that in pooled liver microsomes (7.6 mL/min/mg) suggested that enteric CYP4Fs do not contribute significantly to the initial O-demethylation of pafuramidine during first pass. A role for enteric CYP4F in subsequent O-demethylation reactions remains to be determined. Interestingly, quantitative Western blot analysis of these intestinal preparations indicated appreciable CYP4F protein expression in the small intestine, with a mean (range) of 7 (3 – 18) pmol/mg, which was comparable to that for CYP2C9. This observation suggested that, like CYP2C9, CYP4F could represent an appreciable portion of the human intestinal CYP pie (112).

Other CYPs. Other CYP enzymes shown to be expressed in the human small intestine at the mRNA level include CYP1A2, but only after treatment with

omeprazole (82), CYP1B1 (22), and CYP2C8 and CYP2C18 (93). The importance of these enzymes *in vivo* remains to be determined. By immunoblot analysis, and with prolonged exposure, CYP2A6, CYP2B6, CYP2C8, CYP2E1, and CYP4A11 either were not detected or were expressed in only trace amounts (24,25,112,113). The roles of these enzymes in enteric drug metabolism are likely to be negligible.

Other Phase I Enzymes

Other phase I enzymes reported to be expressed in the human intestine include carboxylesterases (CESs) (114), epoxide hydrolases (24,115), and flavin monooxygenases (FMOs) (116). CESs catalyze the hydrolysis of a variety of ester- and amide-containing compounds (117); epoxide hydrolases catalyze the hydrolysis of epoxides formed via oxidative metabolism, mediated usually by the CYPs (115); and FMOs readily N- and O-oxygenate a variety of drugs and pesticides (116). Of these non-CYP enzymes expressed in the intestine, the CESs have been implicated in the first-pass metabolism of some drugs. Whereas the CES1 family predominates in the liver, the CES2 family predominates in the small intestine (114). Human intestinal microsomes have been shown to catalyze the hydrolysis of betamethasone valerate and aspirin at comparable (aspirin) or greater (betamethasone valerate) rates than human liver microsomes (114). Likewise, intestinal biopsy tissues were as proficient as liver biopsy tissues in converting the prodrug irinotecan to the active chemotherapeutic metabolite, SN-38 (10,11). Approximately one-third of an intravenous radiolabeled dose of irinotecan has been detected in human bile as unchanged drug (118). Therefore, because the bile duct empties into the duodenum, direct conversion of the prodrug to SN-38 could occur in the intestine, as well as bacterial β -glucuronidase-mediated deconjugation of SN-38 glucuronide, leading to accumulation of SN-38 in the intestine and the potential for toxicity (i.e., severe diarrhea). Moreover, the large interindividual variability in the systemic exposure of irinotecan and SN-38 following oral administration of irinotecan has been attributed, in part, to interindividual variation in the extent of intestinal CES-mediated first-pass metabolism (119).

Epoxide hydrolases have been detected in the human small intestine, but protein levels and catalytic activity were much lower ($\leq 6\%$) relative to the liver (24,115). Although a significant role for intestinal epoxide hydrolases in the first-pass metabolism of drugs has not been described, these enzymes could play a protective role in the detoxification of procarcinogenic epoxides generated from environmental xenobiotics (6). Like epoxide hydrolases, flavin monooxygenases (to date only FMO1) have been detected in human small intestine, but the much lower catalytic activity (*p*-tolyl methyl sulfoxidation) relative to the liver (0.11 ± 0.04 vs. 2.8 ± 1.4 nmol/min/mg microsomal protein, respectively) indicates a minimal role for these enzymes in the first-pass metabolism of drugs (116,120).

Phase II Enzymes

Sulfotransferases

Sulfate conjugation via the sulfotransferases (SULTs) is important in the biotransformation of many endogenous compounds, including neurotransmitters and steroid hormones, as well as xenobiotics, including drugs. The nomenclature of these enzymes is similar to that of the CYPs. At least four SULTs are

known to be expressed and to have functional activity in the human GI tract: SULT1A1, SULT1A3, SULT1E1, and SULT2A1. Using cytosolic fractions prepared from the stomach, small intestine, and colon of 23 unrelated organ donors, Chen et al. (121) showed the stomach and colon to have low sulfation activity toward 2-naphthol (SULT1A1) and dopamine (SULT1A3) and to have very low to no activity toward estradiol (SULT1E1) and dehydroepiandrosterone (DHEA) (SULT2A1). Comparatively, sulfation activity toward all probe substrates was higher in the small intestine. Given the much greater surface area of the small intestine, sulfation activity in this section of the GI tract is undoubtedly the most important with respect to drug metabolism. Average ($\pm$ SD) small intestinal SULT1A1 and SULT2A1 activities were less than one-half and approximately one-fifth, respectively, of the corresponding activities measured in four human liver cytosolic preparations (2.1 ± 1.4 vs. 5.3 ± 1.0 nmol/min/mg and 32 ± 33 vs. 140 ± 28 pmol/min/mg, respectively) (121). In contrast, small intestinal SULT1A3 and SULT1E1 activities were approximately threefold higher than and comparable to the corresponding hepatic activities (0.45 ± 0.25 vs. 0.17 ± 0.05 nmol/min/mg and 3.3 ± 0.9 vs. 2.6 ± 1.6 pmol/min/mg, respectively) (121). Intestinal sulfation activity toward all probes substrates showed large interindividual variation, as exemplified by coefficients of variation of at least 60%, consistent with an earlier report involving 62 human jejunal preparations analyzed for SULT1E1 and SULT2A1 immunoreactive protein (122). SULT activity along the length of the small intestine varied among different donors; some donors showed higher activity in the proximal portion, while others showed higher activity in the distal portion (121). Age, sex, underlying pathology, and time of tissue storage appeared not to influence SULT activity and/or protein expression (121,122). No significant correlation was evident between any of these enzymes with respect to catalytic activity or protein expression, suggesting the enzymes are regulated independently (121,122).

Of the aforementioned intestinal SULTs, SULT1A3 and SULT1E1 have been implicated to contribute significantly to the first-pass metabolism of some drugs. Intestinal SULT1A3-mediated metabolism likely contributes to the low F_{oral} of the β -adrenergic agents isoproterenol and terbutaline (7,123,124) (Table 1). SULT1E1 is likely the major intestinal SULT involved in the first-pass metabolism of ethinyl estradiol (8,122,125) (Table 1).

UDP-Glucuronosyl Transferases

Glucuronidation via the UDP-glucuronosyl transferases (UGTs) represents another major conjugative reaction involved in the biotransformation of a variety of exogenous and endogenous compounds. As with the SULTs, the nomenclature for the UGTs is similar to that of the CYPs. In addition to the liver, the UGTs are expressed in a number of extrahepatic tissues, including the GI tract (126,127). Similar to the SULTs, relative to the small intestine, glucuronidation activity, in general, appears to be much lower in the stomach and colon (and esophagus) (126). The expression of a relatively small number of UGTs has been confirmed in the small intestine by multiple laboratories using the same or different approaches: UGT1A1, UGT1A3, UGT1A8, UGT1A10, and UGT2B7 (128). In addition, selective expression of UGT1A8 and UGT1A10 mRNAs in the small intestine and/or colon versus the liver has been reported by multiple investigators (128). Of all these enzymes, only UGT1A1 and UGT2B7 have been detected at the protein level in small intestinal microsomes (127);

specific antibodies are not yet available for the remaining enzymes (128). Using microsomes prepared from the three regions of three unrelated donor intestines, Fisher et al. showed UGT1A1 activity, as measured by estradiol 3-glucuronidation, to be generally much higher than in pooled human liver microsomes (0.2–3.9 vs. 0.4 nmol/min/mg) (127), suggesting an important role for intestinal UGT1A1 in the first-pass metabolism of relevant drug substrates. In contrast, intestinal UGT2B7 activity, as measured by morphine 3-glucuronidation, was at most one-fifth of that measured in the pooled liver microsomes (0–0.5 vs. 2.3 nmol/min/mg), suggesting a minor role for intestinal UGT2B7 in the first-pass metabolism of morphine and other UGT2B7 substrates. Multiple investigators have shown many enteric UGTs to have large interindividual variation in expression level and/or catalytic activity (128,129). Moreover, UGT activity along the length of the small intestine appears to vary with substrate/UGT isoform (128,130). For example, UGT activity toward testosterone (a UGT2B substrate) increased gradually from proximal jejunum to colon, whereas that toward bilirubin (a UGT1A1 substrate) decreased sharply from proximal to distal intestine (128).

Of the aforementioned intestinal UGTs, several of the UGT1As have been implicated to contribute significantly to the extensive first-pass metabolism, and hence low F_{oral} , of some drugs. For example, evidence suggests that enteric UGT1A1, in addition to enteric CYP3A and SULT1E1, may contribute to the first-pass metabolism of ethinyl estradiol (8,9,125) (Table 1). The intestine-specific forms, UGT1A8 and UGT1A10, likely are major contributors to the low F_{oral} of raloxifene (13,131,132) (Table 1). Enteric UGT1As (e.g., UGT1A1, UGT1A3) may influence the efficiency of the enterohepatic cycling of SN-38 (133) and ezetimibe (134,135).

Other Phase II Enzymes

Other phase II enzymes that have been identified in the human GI tract include members of the N-acetyltransferase (NAT) and glutathione S-transferase (GST) families (24,129,136–138). Mesalazine (5-aminosalicylic acid), indicated for the treatment of inflammatory bowel disease, undergoes extensive first-pass acetylation, and intestinal NAT, most likely NAT1 (137), is believed to contribute to this process (139). Although both NAT1 and NAT2 have been detected, and to have functional activity in the small intestine, NAT1 activity, as measured by *p*-aminobenzoic acid acetylation, was always higher than NAT2 activity, as measured by sulfamethazine acetylation (137). Moreover, the ratio of NAT1:NAT2 activities varied from 2- to 70-fold. Among four human donor small intestines, NAT1 activity was relatively uniform or increased slightly, whereas NAT2 activity tended to decrease, from the duodenum to the rectum.

The GSTs are commonly implicated in the detoxification or bioactivation of environmental toxins, carcinogens, and some chemotherapeutic agents. Using cytosolic fractions prepared from the GI tracts (stomach to colon) of 16 organ donors, Coles et al. showed GSTP1, GSTA1, and GSTA2 to be the major GST proteins expressed in the small intestine (138). For all three of these enzymes, large interindividual variation was observed in all regions of the GI tract. Despite the high degree of interindividual variability, consistent patterns of expression along the length of the GI tract were evident. Specifically, GSTP1 was expressed throughout the GI tract and decreased progressively from stomach to colon. In contrast, GSTA1 and GSTA2 were expressed at very low levels in the

stomach and colon relative to the small intestinal regions, where levels were high in the duodenum and decreased to distal ileum. Similar differences in expression between stomach and duodenum for GST1 and GSTP were reported by other investigators who examined antral and duodenal biopsy specimens obtained from 202 patients (140). It has been speculated that the low levels of GSTA in the stomach and colon contribute to the greater susceptibility of these GI tissues to some cancers compared to the small intestine (129,138). With respect to chemotherapeutic agents, Gibbs et al., using cytosol prepared from 12 small intestines and 23 livers, reported comparable busulfan conjugation intrinsic clearances (GSTA activity) between the two organs (0.17 ± 0.07 vs. 0.18 ± 0.09 $\mu\text{L}/\text{min}/\text{mg}$), suggesting a role for intestinal GSTA in the first-pass metabolism of busulfan (136).

SUMMARY AND PERSPECTIVE

The majority of drugs are taken orally. Accordingly, for those intended to act systemically, a significant fraction of the dose can be eliminated during its first passage through a sequence of organs prior to entering the systemic circulation. For some drugs, the extent of first-pass elimination can be large enough such that F_{oral} is reduced significantly, with the consequent potential for a reduced clinical response. Next to the liver, the small intestine can represent a major organ of first-pass drug elimination, the means of which occurs primarily via metabolism.

Like the liver, the small intestinal mucosa is replete with a myriad of drug biotransformation enzymes, including both phase I and phase II enzymes. Of all of these enzymes, the CYPs are the most extensively studied. Of the CYP enzymes, CYP3A is the most extensively studied and represents, on average, approximately 80% of total immunquantified CYP content in the proximal human small intestine. In addition, microsomal CYP3A catalytic activity and immunoreactive protein content in the proximal region (duodenum to mid-jejunum) are within the ranges reported for human liver microsomes. These in vitro observations are consistent with clinical studies demonstrating that the intestinal contribution to the low and variable F_{oral} of some CYP3A substrates can rival the hepatic contribution. However, because intestinal and hepatic CYP3A appear to be regulated independently, and thus do not correlate, CYP3A activity measured in one organ will not necessarily predict CYP3A activity in the other. Taken together, the development/refinement of in vivo methods capable of delineating intestinal from hepatic first-pass metabolism, as well as capable of delineating CYP3A-mediated metabolism from transporter-mediated efflux, is of clinical importance. This is an ongoing and active area of research, as the successful prediction of intestinal first-pass metabolism could aid in the therapeutic management of drugs with a low and variable F_{oral} , particularly those with a narrow therapeutic window.

Other human enteric CYP enzymes have been identified and characterized in vitro (CYP1A1, CYP2C9, CYP2C19, CYP2D6, CYP2J2, CYP4F), but their role in drug disposition in vivo remains to be determined. Regarding other enteric phase I enzymes, carboxylesterases have been implicated in the first-pass metabolism of some drugs, whereas roles for the epoxide hydrolases and FMOs remain to be determined. Regarding phase II enzymes, while a number such families have been known to be expressed in the human intestine for some time

(e.g., SULTs, UGTs, NATs, GSTs), progress on the identification and quantification of individual isoforms has lagged behind that of the CYPs. With the increasing availability of quality human intestinal tissue, along with the ongoing identification of selective probe substrates, inhibitors, and antibodies, it is anticipated that a comprehensive characterization of these enzymes will soon be possible. Meanwhile, further refinement of human intestinal cell culture models and/or the identification of an appropriate animal model should improve our understanding of the unique nature of intestinal drug-metabolizing enzymes and allow us not only to better predict the impact of the intestine on the overall first-pass elimination of existing drugs but also to improve the drug selection process during preclinical development.

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Food Effects on Drug Absorption and Dosage Form Performance

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INTRODUCTION

The gastrointestinal (GI) tract is designed to make sure that the body gets the maximal benefit out of nutrients in the ingested food. The main role of the GI tract is to digest foodstuffs and absorb nutrients, vitamins, and electrolytes, while at the same time to exclude and metabolize ingested xenobiotics that could be potentially harmful. Intake of a meal induces a whole range of changes in the GI tract mediated by physical, nervous, and endocrine pathways. These changes include (i) increased volume due to GI secretions, (ii) increased gastric residence time, (iii) increased peristaltic movements, (iv) changes in gastric and intestinal pH, (v) further changes in luminal composition due to secretion of bile and pancreatic fluids, (vi) alterations in luminal metabolism, (vii) and increased perfusion of the GI mucosa and the liver. Many of these changes can impact the absorption and pharmacokinetics of drugs. The mechanisms behind this are manifold and depend on the physicochemical characteristics of the drug as well as the physiological changes.

The oral route is the most desirable method for drug administration. This is due to ease of administration, lack of necessity for involvement of health care personnel, and the relative ease of production of oral dosage forms. However, orally ingested drugs are also challenged by the nature of the GI tract, which will try to eliminate any potentially harmful compound. Therein lies an inherent contradiction, in that most drugs can be considered to be xenobiotics, but despite this it is desirable to have them absorbed.

The oral bioavailability of many, but not all, drugs is affected by food intake. The absorption of a drug can be increased, decreased, delayed, or accelerated (Fig. 1), depending not only on the physicochemical characteristics of the drug compound but also on the type of dosage form, composition of the ingested food, and general state of the GI tract. This complex matrix of cause and effect complicates the elucidation of the food effect on a given drug. Clinically, significant interactions are typically assessed in terms of peak plasma drug concentration ($C_{\max}$), time to $C_{\max}$ ($T_{\max}$), and area under the concentration-time curve (AUC).

In recent years, food effects on drug absorption and food-drug interactions have been the subjects of several reviews (1–5). In the present chapter, the physiological changes induced by food intake will be reviewed. Then the food effect will be addressed as a function of drug characteristics as well as food type. Finally, food effects on the various types of dosage forms will be described and consideration will be given to the different formulation approaches that can be employed to circumvent or, at least, minimize food effects.

PHYSIOLOGICAL EFFECTS OF FOOD INTAKE

Digestion is initiated by reduction of the particle size of food in the mouth by chewing, which also mixes the food with saliva and initiates the enzymatic

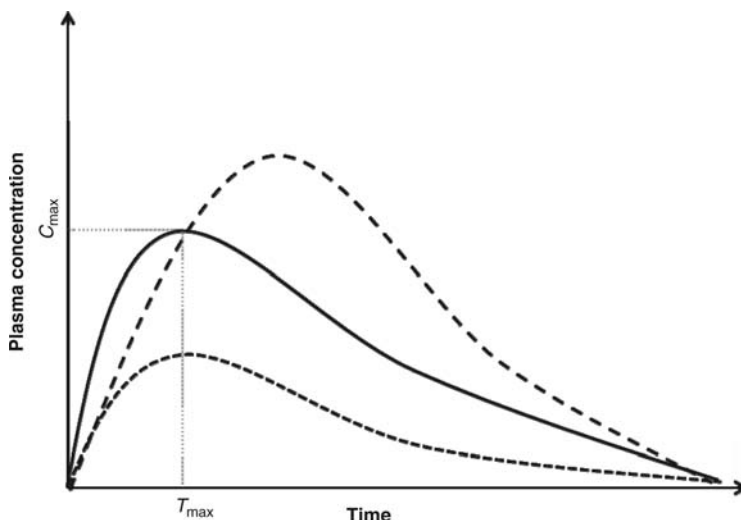


FIGURE 1 Examples of plasma curves after drug intake in the fasted state (—), and in the fed state for a drug showing positive food effect (---) and negative food effect (· · · · ·).

degradation of carbohydrates by salivary amylase. Digestion in the stomach is mediated by gastric acid and by enzymatic digestion. Proteins and lipids are especially prone to enzymatic digestion in the stomach. In the stomach, the low pH not only kills many microorganisms but also degrades acid sensitive compounds.

Immediately after intake, food interrupts the migrating motor complex (MMC) that controls the motility pattern of the stomach in the fasted state (see chaps. 1 and 3).

The stomach serves as a reservoir that enables a rather quick intake of a large meal; the volume of the stomach can expand up to 1.5 L. The ingested food will be subjected to the muscular contractions of the stomach and gastric secretions. The muscular contractions disperse the food particles and grind them into a suspension with a softer and more liquid consistency. The dispersion of solid foods has recently been comprehensively reviewed (6). Food intake induces the secretion of the hormone gastrin in the stomach, which in turn stimulates gastric acid secretion from the parietal cells along with the secretion of gastric lipase from the chief cells in the gastric mucosa. Pepsinogen is secreted by the chief cells and is subsequently hydrolyzed to the active enzyme pepsin by gastric acid. This results in fat and protein digestion being initiated in the stomach. Gastric lipase is responsible for approximately 10% to 20% of dietary triacylglyceride (TAG) hydrolysis (7). Gastric lipase removes one fatty acid from a TAG molecule, thus forming diacylglycerides (DAG) and free fatty acid (FFA). Both FFA and DAG have surface-active properties and facilitate emulsification of TAG. In addition, intragastric lipid digestion has been shown to facilitate the drug solubilization process *in vitro* (8).

Upon arrival in the stomach the food is met by a low gastric pH, which is then buffered by the various food components and, depending on the composition of the solid foods, especially the fluids ingested with the meal, will increase temporarily to varying extents. In the fasted state, the pH of the

stomach fluids is in the 1.3 to 2.5 range, but after food intake the pH can easily increase to a value in the 4.5 to 5.8 range. Over time, with secretion of gastric juices and following gastric emptying, values will return to those of the fasted state. The time to return to fasted state values has been reported to be between one and four hours, depending on the composition and volume of the ingested meal (9–11). The presence of food in the stomach increases the gastric retention time. This is necessary to enable an appropriate start to digestion in the stomach, including liquefying of the foodstuffs, on the one hand, and to prevent overloading of the intestinal digestive capacity with too much food, on the other hand. The pylorus is a muscle that divides the stomach and the duodenum. Contractions of the pylorus control the gastric emptying; this process can run over several hours. Gastric emptying is controlled by both nervous and endocrine pathways. Food intake and presence of chyme in the duodenum lead to gastric inhibitory impulses in the enteric nervous system, with these impulses being referred to as the enterogastric reflex. Furthermore, enteric hormones such as cholecystokinin and secretin are released from cells in the duodenum and contribute to the suppression of gastric emptying.

The emptying rate of the stomach is primarily a function of the rate at which calories enter the duodenum, but it is also influenced by other factors like volume, pH, osmolality, viscosity, and temperature. The caloric output from the stomach has been found to be between 2 and 4 kcal/min (8.4 and 16.4 kJ/min). Since fat contains twice as many calories per gram as carbohydrates and proteins, the fat content of a meal plays a critical role in determining the gastric emptying rate (12). For example, administration of a lipid-rich meal with 200 mL of water resulted in a lag time of gastric emptying of 44 ± 20 minutes, compared with a lag time of 14 ± 11 minutes after intake of 200 mL water (13).

Liquids are emptied faster than solid food items, mainly because the pylorus holds back larger food particles until further particle size reduction has taken place. Liquids are emptied at a rate that is a linear function of the square root of the volume (12,14), while the emptying solid food items show a lag time before emptying of the stomach is initiated at a constant rate (15,16). Indigestible particles, approximately 3 to 4 mm in diameter (and perhaps even up to 7 mm in certain cases), can pass the pylorus, while larger nondegradable particles will have to wait for the MMC phase III before leaving the stomach (17).

The impact of food on gastric emptying can be assessed by scintigraphic methods or by appearance of certain drugs in plasma. By use of scintigraphic methods, gastric emptying time of nondisintegrating labeled tablets administered with and without a breakfast meal has been assessed (18). In fasted state, the median gastric emptying time was 37 minutes with an interquartile range of 19 to 74 minutes. The corresponding figures in the fed state were 149 minutes and 119 to 171 minutes, reflecting that the nondisintegrating tablet was emptied most probably with the MMC housekeeper wave, which returns only after all food has been emptied from the stomach. Drug markers of gastric emptying include paracetamol, which is absorbed immediately after it enters the duodenum and has been shown to be a good marker for gastric emptying (19).

Intestinal Events

The chyme is transferred from the stomach in a well-controlled manner to the duodenum, which is the first part of the small intestine, approximately 12 fingers

long (duodenum is derived from the Greek *dodekadaktylon*, meaning “12 fingers”). In the duodenum, bile and pancreatic juices are secreted, leading to further dilution, dispersion, and digestion of the food components. During transit through the rest of the small intestine (jejunum and ileum), food components will be further degraded and absorbed across the intestinal membrane into the portal and subsequently into the systemic circulation. One important observation is that the small intestine is not a liquid-filled tube; substances do not move uniformly through the digestive system and thus do not necessarily leave segments of the digestive tube in the same order as they arrive (20). At the same time, total transit time in the small intestine is fairly constant and has been reported to be between three and four hours (13,18). Components from the food that are not absorbed will enter the colon. The main function of the colon is to absorb water and form the stools, but some drug compounds can also be absorbed in the colon.

In the duodenum, cholecystokinin stimulates contraction of the gallbladder and pancreatic secretion of enzymes, whereas secretin stimulates the secretion of bicarbonate-containing juice by the pancreas. Several publications have characterized the duodenal and jejunal fluids in the fed and fasted states. Some of these references are summarized in Table 1 (10,21–35). For the fed state, it should be noted that very different meals and different procedures (e.g., time of sampling and analytical method) have been applied, and these differences are reflected in the rather large variation in the data.

The bile and pancreatic secretions are both slightly alkaline, while the chyme arriving from the stomach has a lower pH, leading to an overall decrease in the pH in the intestinal fluids, from 6.2 to 7.5 in the fasted state to 5.7 to 6.6 in the fed state (Table 1). As can be seen from Table 1, there is a tendency to an increase in pH when moving from the duodenum to the jejunum in the fasted state. Because of the lack of data, this tendency has not been confirmed for the fed state.

Osmolality, generally, increases in the fed state as a consequence of chyme and intestinal secretions, but the increase will also depend on the type of food and time of sampling (Table 1). Surface tension, however, does not change significantly between the fasted and fed state. The intestinal buffer capacity is also an interesting parameter for characterizing the intestinal fluids, though it has been measured in only a few studies. Kalantzi et al. (10) found a median buffer capacity of 5.6 mmol/L/pH in the fasted state, increasing to values of 18 to 30 mmol/L/pH in jejunal fluids in the fed state.

In the fasted state, bile salt (BS) levels in the small intestine range from 1.5 to 6 mM, with most levels being reported around 2.5 mM. Phospholipids (PL) are secreted together with BS in the bile, but only a few studies have been carried out on the PL content in the fasted small intestine (Table 1). Food-induced gallbladder contraction results in an increase in BS level to between 8 and 20 mM, with no well-documented difference between the duodenal and jejunal content. The molar ratio between BS and PL in the fed state will also be dependent on the PL level in the food and has been reported to be in the range of 2 and 16, with the majority of ratios between 2 and 4 (Table 1).

The function of the bile is to solubilize lipophilic components in the food and help the emulsification processes to make TAGs available for digestion by lipases from the pancreatic juice. Pancreatic TAG lipase is by far the most important lipase in TAG digestion. It is an sn1,3-specific lipase and, thus, releases monoacylglycerol (MAG) and FFAs. The challenge for the pancreatic

TABLE 1 Characteristics of the human intestinal fluid (mean ± SD)

Region	Total BS (mM)	Total PL (mM)	Ratio (BS/PL)	pH	Surface tension (mN/m)	Osmolality mOsm/kg	Reference
Fasted state							
Duodenum (n = 7)	5.9 ± 1.8	—	—	6.8	—	—	(21)
Duodenum (n = 4)	3.5 ± 1.8	0.1 ± 0.1	39	6.5 ± 0.5	—	—	(34)
Duodenum (n = 15)	2.6	—	—	6.2	32.3	178	(10)
Duodenum (n = 12)	2.82	—	—	6.7	33.6	197	(10)
Duodenum (n = 6)	2.6 ± 1.6	—	—	7.0 ± 0.4	—	137 ± 54	(29)
Duodenum (n = 7)	2.5	0.4	6	—	—	—	(25)
Duodenum (n = 5)	2.7	0.6	4.5	6.6	41.2	224	(35)
Jejunum (n = 37)	2.9 ± 2.9	—	—	7.1 ± 0.6	—	271 ± 15	(26)
Jejunum (n = 10)	1.5 ± 1.8	—	—	6.7 ± 0.9	33.7 ± 2.8	278 ± 16	(28)
Jejunum (n = 6)	3.5 ± 1.6	—	—	6.8 ± 0.4	—	200 ± 68	(29)
Jejunum (n = 3)	2 ± 0.2	0.2 ± 0.07	10	7.5	28 ± 1	—	(30)
Jejunum (n = 6)	—	—	6	—	—	—	(31)
Fed state							
Duodenum (n = 7)	13.4 ± 4.3	1.9 ± 0.4	9.6	6.4	—	—	(21)
Duodenum (n = 5)	14.5 ± 8.8	4.8 ± 1.8	3	—	—	—	(24)
Duodenum (n = 6)	9.3 ± 0.8	2.4 ± 0.35	3.9	5.7	—	—	(27)
Duodenum (n = 12)	11.8	4.31	2.7	6.5	27.8	416	(10)
Duodenum (n = 8)	24	1.5	16	—	—	—	(25)
Duodenum (n = 5)	3.6	1.8	2	5.9	35	285	(35)
Duodenum (n = 5)	5.2	1.2	4.3	6.1	35	278	(35)
Jejunum (n = 3)	8 ± 0.1	3 ± 0.3	2.7	6.1	27 ± 1	—	(30)
Jejunum (n = 15)	12	—	—	6.6	28	400	(10)
Jejunum (n = 6)	0.5–8.6	0.1–3.9	1–3	—	—	—	(31)
Jejunum (n = 13)	16.19 ± 1.51	—	—	—	—	—	(32)
Jejunum (n = 16)	15	—	—	—	—	—	(33)

lipase is to reach the interface between the TAG core of the emulsion particle and its surface, which may already be covered with a mono- or multilamellar layers of MG and FFA arising from gastric lipolysis (36). For this purpose, the pancreatic colipase plays an important role. Colipase is believed to clear the surface of the emulsion particles and anchor the lipase to the TAG interface, where it is active (37,38). Other relevant lipases in the pancreatic juice are carboxyl ester hydrolase and pancreatic lipase-related protein 2, both of which show a broader specificity than the pancreatic triglyceride lipase and hydrolyse cholesterol esters and PL (37,38).

Intestinal lipid digestion is a dynamic process (24,39,40), involving the formation of different colloid phases that in complex ways are transformed into mixed micelles contain BS, PL, FFA and MAG. The mixed micelles diffuse to the unstirred water layer covering the epithelium, where they dissociate into monomers. The FFA and monoacylglyceride are then absorbed. At any given time during digestion, a very complex mixture of different colloid phases will be present in the GI tract. Lipid digestion primarily takes place in the upper part of the small intestine. BS are actively absorbed in the lower ileum and return to the liver, from where they can be resecreted in the bile, with the whole process being referred to as enterohepatic circulation.

In addition to the lipases mentioned above, the pancreatic juice also contains proteases and amylases. The activities of these enzymes also contribute to the continuously changing intestinal environment during digestion.

Various food components are substrates for intestinal absorptive transporters, efflux transporters, and metabolizing enzymes, and can therefore impact their activity (41,42). Food items that contain phytochemicals, such as fruit, vegetables, tea, herbs, and spices, are especially likely to induce effects on metabolizing enzymes or efflux transporters. Examples of food items that contain inhibitors of cytochrome P450 3A4 are grapefruit juice, garlic, and red wine (42), while efflux mediated by P-glycoprotein (Pgp) has been shown to be inhibited by green tea, rosemary, and some fruit extracts containing inhibitors of cytochrome P450 3A4 (41). In addition, some endogenous constituents of the intestinal fluids, for example, bile salts and phospholipids, have been shown to inhibit efflux mechanisms (43–45).

Food intake also induces an increased splanchnic blood flow, which in turn will increase the absorption and transfer of nutrients into the blood. Amino acids, small peptides, and monosaccharides derived from hydrophilic nutrients like proteins and carbohydrates are absorbed via the portal blood into the systemic circulation.

Lipophilic nutrients like lipids and lipid-soluble vitamins are transported via the lymphatic system. After uptake of FFAs and MAGs into the enterocyte, TAGs are reformed and participate in the formation of chylomicrons. The chylomicrons are transported via the lymph vessels directly to the systemic circulation, circumventing first-pass metabolism in the liver.

FOOD EFFECTS AND DRUG CHARACTERISTICS

As described above, food intake induces many changes and complex reactions in the GI tract. Some of these will impact the bioavailability of a given drug substance, with the extent of the effect dependent on its physicochemical characteristics. Important parameters are solubility, pK_a , $\log D$, and acid-base properties.

Small molecular weight drug compounds can be categorized in the Biopharmaceutical Classification System (BCS) according to their solubility and

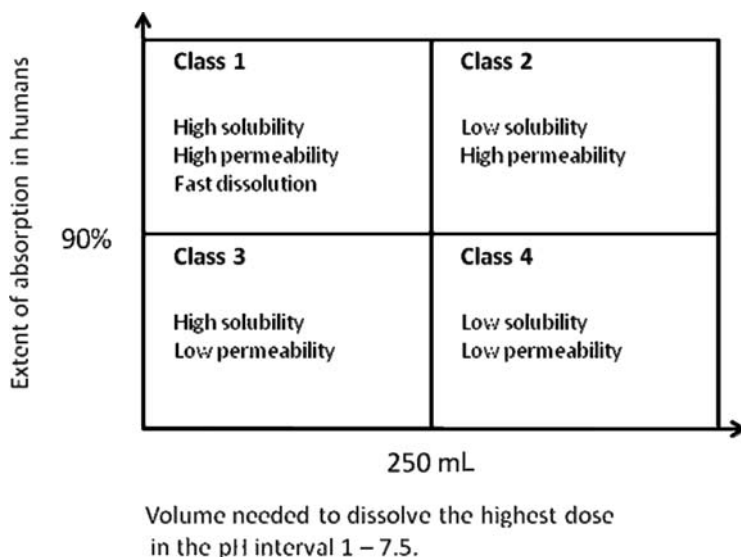


FIGURE 2 The Biopharmaceutics Classification System (BCS) as defined by the FDA and Amidon et al., 1995 (46).

permeability, as depicted in Figure 2 (46). The BCS has been expanded into the Biopharmaceutical Drug Disposition Classification System (BDDCS) by additionally taking metabolism, uptake, and efflux transporter affinity of the drug into account (47). Figure 3 summarizes the BDDCS. Since all the BCS and BDDCS parameters are impacted by the physiological effects of food, as described above, it makes sense to classify drug-food interactions in terms of the BDDCS. This approach has previously been used by other authors (2,48) and is summarized in Figure 4.

In the following discussion, the food effect on drugs according to their BDDCS classification, primarily dosed in immediate-release (IR) dosage forms, will be reviewed. However, it should be noted that not only the drug but also the dosage form can contribute to the food effect. Abrahamsson et al. showed that the disintegration of IR tablets in dogs is delayed upon coadministration with food (49). In agreement with this by simultaneous assessment of intraluminal and plasma drug concentrations Brouwers et al. demonstrated that the delayed $t_{\max}$ of amprenavir in the fed state in humans may be due to delayed gastric tablet disintegration (22).

BCS Class 1 Drugs

These drugs are highly soluble and permeable while characterized by their tendency to extensive metabolism, but lack of transporter effects on absorption, as shown in clinical studies. Class 1 drugs are small hydrophilic compounds that are well absorbed in the GI tract and often show no food effect on bioavailability. Accordingly, no food effect on AUC has been found for albuterol, diazepam, or verapamil (50–52). A survey of 30 class 1 drugs revealed that 97% showed no (67%) or a negative (30%) food effect on AUC (53). The negative food effects not only could be associated with interactions between drug and food components but could also be due to delayed gastric disintegration of the IR formulation (49).

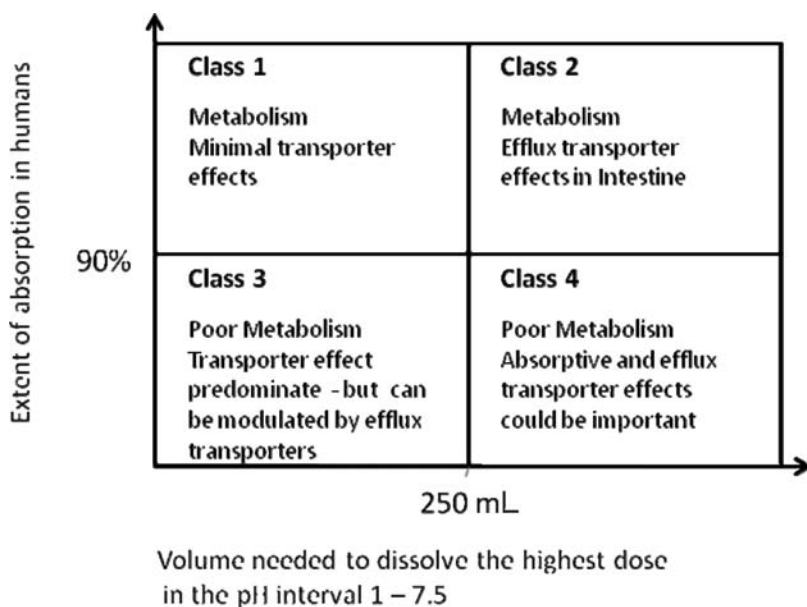


FIGURE 3 The Biopharmaceutics Drug Disposition Classification System (BDDCS) according to Wu and Benet, 2005 (47).

Accordingly, several papers have observed a negative food effect on acetaminophen (paracetamol) in IR tablets, expressed as a delayed $T_{\max}$ and a decreased $C_{\max}$, without impacting the AUC, while no such effects were observed for solution dosage forms (49,54–56).

The prevailing food effect of class 1 compound is a delayed absorption, that is, increased $T_{\max}$, because the rate of absorption is often controlled by gastric emptying. Even when class 1 drugs have shown to be substrates for transporters in vitro, the high solubility in the GI tract is likely to result in saturation of transporters, and their contribution to transport in vivo will be suppressed. This has been evidenced by studies with midazolam (57).

Class 1 drugs are frequently good candidates for controlled release formulations and, in this case, dosage form-related food effects on absorption can be relevant (see sect. “Food Effect and Dosage Form”).

BCS Class 2 Drugs

For BCS class 2 drugs, solubility or dissolution rate in the GI tract is the rate-limiting step for absorption. In addition, they are often characterized by extensive metabolism and efflux transport. Absorptive transporters are generally not important in the intestine for these compounds, but their uptake into the liver may be mediated by transporters (47).

The physiological changes induced by food intake that can increase the solubility and/or dissolution rate of class 2 drugs will potentially increase the bioavailability, while the slower gastric emptying will often result in less variability in plasma levels compared with that in the fasted state (2). The increased

	High Solubility	Low Solubility
Extensive Metabolism	<u>Class 1</u> $F_{\text{extent}} \leftrightarrow$ $T_{\text{max}} \uparrow$	<u>Class 2</u> $F_{\text{extent}} \uparrow$ $T_{\text{max}} \uparrow \downarrow \leftrightarrow$
Poor Metabolism	<u>Class 3</u> $F_{\text{extent}} \downarrow$ $T_{\text{max}} \uparrow$	<u>Class 4</u> $F_{\text{extent}} \uparrow \downarrow \leftrightarrow$ $T_{\text{max}} \uparrow \downarrow \leftrightarrow$

FIGURE 4 Predicted effect of a high-fat meal according to BDDCS class. *Source:* From Ref. 48.

GI volume, from food and from induction of gastric and intestinal secretions, will increase the capacity to dissolve the drug and facilitate the rate of dissolution. Most importantly, the changes in luminal composition increase the solubilization capacity of the fed state GI fluids (10,30). This is due in large part to BS and PL in the bile, but the food components themselves and their enzymatic degradation products also play a considerable role. For example, the presence of FFA and MAG from TAG hydrolysis has a significant impact on the solubility of many class 2 compounds (13,58,59).

Food-induced pH changes will also impact dissolution and thus absorption of poorly soluble weak acids and bases. The increased postprandial gastric pH will be of particular benefit for the dissolution of weak acids with pK_a lower than about 5, for example, ketoprofen. For weakly basic drugs having pK_a near the fed state gastric pH values of 4.5 to 5.8, dissolution rate and solubility can be decreased in the postprandial state, for example, albendazole.

Intake of a lipid-rich meal will lead to formation of chylomicrons in the enterocytes. Chylomicrons are responsible for the transport of lipids and lipid-soluble vitamins from the intestine to the blood via the lymphatic system. Highly lipophilic drugs are incorporated into the chylomicrons and are transported in the lymph until entrance into the systemic circulation by the left subclavian vein. By employing this route, the lymphatically transported drug bypasses the first-pass metabolism in the liver, thereby increasing the bioavailability.

In conclusion, many postprandial changes, alone or in combination, can contribute to an increased bioavailability of class 2 compounds. A review of 28 class 2 compounds found that 20 (71%) had positive food effect (on AUC), while 8 (29%) had no food effect (53).

Several publications have attempted to elucidate the mechanism of the changed AUC. The bioavailability of danazol (log P 4.6) in humans was increased four times by intake of a lipid-rich meal (13). $C_{\max}$ and $T_{\max}$ increased 2.5 and 3.3 times, respectively. The increased bioavailability in the postprandial state was explained by the combination of the increased gastric dissolution time (due to reduced gastric emptying rate) with the increased solubilizing capacity of the fed intestine. A lipophilic drug, halofantrine hydrochloride (log P 8.5 of the base), was showed to have an approximately threefold higher bioavailability after coadministration of a lipid-rich meal to humans, whereas $C_{\max}$ was increased 6.6 times (60). Further, the $T_{\max}$ was decreased twofold. It was suggested that the increased bioavailability was caused partly by increased solubility and dissolution in the GI lumen (61) and partly by incorporation of the drug with lipoproteins in the intestinal cells and subsequent transport via the lymph. Reduced drug clearance due to association with lipoproteins in blood may also play a role (62).

Bioavailability of the poorly soluble drug probucol (log P 10.0) was increased from 2.5% to 5.8% by coadministration with a fatty meal (50% energy from fat) in a mini-pig study. The increase was caused by 2.9-fold increase in both $C_{\max}$ and AUC, while no changes in $T_{\max}$ were seen.

Similarly, intake of the FDA recommended meal induces a 1.6-fold increase in both AUC and $C_{\max}$ of griseofulvin from an IR formulation (63).

The weak base protein inhibitor indinavir is an atypical example of a class 2 drug, in that it does not show positive food effect. However, indinavir has two pK_a values, at 3.7 and 5.9, so it is likely that the increase in gastric pH that is induced by food intake suppresses dissolution during gastric residence, leading to a reduced absorption (2).

BCS Class 3 Drugs

Class 3 drugs have a high solubility but a low permeability. Their uptake often involves absorptive and efflux transporters. They tend not to be metabolized, so renal or biliary clearance of unchanged drug is an important mechanism for their elimination.

Since many components in food, and also endogenous substances from bile, have been shown to competitively inhibit the intestinal transporter function, this can lead to a reduced uptake of class 3 compounds in the fed state (48). A survey of 23 class 3 compounds showed that 14 had a negative food effect, 7 had no effect, while only 2 had a positive food effect (on AUC) (53).

Fexofenadine is an example of a class 3 drug that shows a negative food effect because of poor intestinal permeability (64). Another example is alendronate, which is basically not absorbed when taken with food. The absorption is decreased 60% with coffee or juice. Absorption is optimal if taken two hours before breakfast, to avoid contact with potential complexing agents in the gut lumen (65).

BSC Class 4 Drugs

These drugs are characterized by both a low solubility and a low permeability. They tend to have low metabolism, but their uptake can be affected by both absorptive and efflux transporters. While the intestinal solubility and dissolution rate might be improved in the fed state and efflux transporters are inhibited,

the inhibition of absorptive transporters can reduce the absorption of a class 4 drug. A standard breakfast prior to furosemide administration resulted in a 30% decrease in bioavailability (66). On the other hand, digoxin, which is recognized to be a Pgp substrate, shows an increased bioavailability in the fed state (67). Complexation with components in the food can decrease absorption of BCS class 4 compounds. Ciprofloxacin forms an insoluble complex with Ca in some dairy products; metal ions such as Ca, Fe, and Mg in dietary supplements; and Ca, Al, and/or Mg in antacids, decreasing drug absorption (68).

FOOD EFFECT AND FOOD COMPOSITION

As described above, the effect of food will also depend on the kind of food that has been ingested. A lipid-rich meal will induce larger changes in the physiology; food will be retained longer in the stomach and more bile and pancreatic juice will be secreted. The amount of the food that is necessary to initiate the fed state is not very well elucidated. Administration of a small amount, 2 g, of long chain MAG to humans was observed to change gastric emptying, although not to a statistically significant degree, and also increased the levels of BS, PL, and cholesterol in the duodenal fluids, indicating that bile secretion had been stimulated (25). In addition, the exact timing of drug intake in relation to a meal is also important.

In 2002, the FDA issued a guideline for clinical studies of food effect titled "Food-effect bioavailability and fed bioequivalence studies" (69). The guideline recommends the use of a high-fat meal containing 50% to 65% of energy from lipid, 25% to 30% from carbohydrates, and 15% to 20% from proteins, with the meal providing a total of 800 to 1000 kcal. The reason for recommending this meal is the expectation of a maximum effect on GI physiology and therefore on drug absorption. A possible composition of the meal would be two slices of toast with butter, two eggs fried in butter, two strips of bacon, 4 oz of hash brown potatoes, and 8 oz of whole milk. The breakfast meal is to be ingested after an overnight fast of at least 10 hour. The meal should be eaten in 30 minutes or less, and at 30 minutes the drug product should be administered together with 240 mL of water. One hour after administration, water ad libitum is allowed and four-hour postdose food is allowed.

A study in humans showed that bioavailability of atovaquone (log P 5.07) was unchanged by intake of two slices of toast, but increased 3- to 3.9-fold by further administration of 28 and 56 g of butter, respectively (70). $C_{\max}$ increased 3.9- and 5.6-fold. Similarly, the bioavailability of griseofulvin (log P 1.9) in humans was unchanged by intake of protein-rich or carbohydrate-rich, low-fat meals, but increased approximately seven times by intake of a lipid-rich meal (71). The absorption of cefuroxime axetil is also increased when administered with food (72). The administration of cefuroxime axetil with bread and soup (poori and dal fry) significantly enhanced AUC and $C_{\max}$ when compared with that in lentil-rice cakes and chutney (73).

FOOD EFFECT AND DOSAGE FORM

In the fasted state, the gastric emptying of nondisintegrating tablets, for example, matrix or coated tablets with modified release, typically occurs during the MMC phase III; the housekeeper wave. This results in a large variation in the gastric emptying time, since the MMC cycle usually occurs over a two-hour period and timing of the dose is random within the cycle (18,74).

In the case of postprandial intake of a nondisintegrating dosage form, there is still no agreement on whether the dosage form is emptied from the stomach together with the food, or will reside in the stomach until the next MMC phase III occurs. Some studies have shown that particle up to 3 to 4 mm and maybe even 7 mm are emptied with food (17), while others have shown that particles of both 3 and 10 mm empty from the stomach only *after* emptying of a solid meal is complete (75). The discrepancy might in part be due to the use of different methods for gastric emptying assessment (e.g., γ -scintigraphy, electrical impedance tomography, and radiopaque markers).

Another issue to take into account with regard to nondisintegrating modified release dosage forms is the contraction pattern of the stomach in the fed state. The purpose of these contractions is to homogenize the food, but they can also lead to nonintended erosion or crushing of the modified release matrix formulations, resulting in dose dumping and/or increased bioavailability of the drug (74,76).

As previously noted, the delayed gastric emptying in the fed state can lead to increased $T_{\max}$. In addition, dissolution in the stomach from disintegrating dosage forms has also been shown to be delayed in the fed state, possibly due to delayed tablet disintegration (22,56,77). Studies of tablet disintegration in fed dogs and in vitro using gastric media containing a homogenized meal or a nutritional drink have shown that the delayed intragastric disintegration is most likely due to the formation of a proteinaceous film on the tablet (49,56,77). However, both human and dog studies have shown that for a class 1 drug (paracetamol) in IR tablets, gastric emptying is still the rate-controlling step to absorption (77,78). In contrast, delayed gastric disintegration and/or dissolution resulted in delayed absorption of a class 2 drug (fosamprenavir) from IR tablets (22). Another class 2 drug (felodipine) in an ER formulation also showed a delayed absorption when administered after a breakfast meal. In this case, the food effect was explained by the increased residence time of the drug-releasing ER tablet in the upper part of the stomach, resulting in a delay, followed by a high absorption upon gastric emptying (79).

In general, the small intestinal transit time is in the range of three to four hours and unaffected by food (see above). However, when a nondisintegrating tablet was administered 45 minutes prior to a standard breakfast, the small intestinal transit time was reduced to approximately 100 minutes in those subjects where the tablet had already entered the small intestine before the breakfast was given. This phenomenon is due to the emptying of the distal intestine at the start of food ingestion (18).

In addition, it has been reported that some poorly absorbed pharmaceutical excipients (e.g., mannitol and PEG 400) can decrease the small intestinal transit time via an osmotic effect if given in sufficient amounts. The reduced transit time can result in decreased bioavailability (80,81). However, studies that have demonstrated that these effects have typically used excipient amounts far in excess of usual amounts used in oral solid dosage forms.

FORMULATING TO ELIMINATE THE FOOD EFFECT

The food effect on oral dosage forms can be reduced by optimization of the formulation. A reduced food effect is most easily achieved when the limiting factor for drug absorption is the dissolution rate or intestinal solubility of the

drug. One way of limiting the food effect in this case can be to deliver the drug in a lipid-based drug delivery system, for example, self-microemulsifying drug delivery systems (SMEDDS). The SMEDDS is dosed as a concentrate and will form a microemulsion upon dispersion in the GI tract. A SMEDDS will deliver the drug in solution in a lipid phase and, instead of a dissolution process, the drug will need to be transferred between different colloid phases in the intestine before it encounters the intestinal membrane and is absorbed. The first, and so far most successful, SMEDDS on the market, the cyclosporine A formulation Sandimmune Neoral[®] was developed on the principle of eliminating food effect, and was successful in this regard (82). Charman et al. showed in a human study that the bioavailability of danazol (log *P* 4.2) formulated in a hard gelatin capsule was increased more than threefold by intake of a meal, and $C_{\max}$ was increased threefold (83). By contrast, the bioavailability or $C_{\max}$ using a crude lipid emulsion was not significantly affected by food. However, eliminating the food effect by the use of SMEDDS is not always straightforward. In a study by Perlman et al., the food effect in dogs on five different SMEDDS containing torcetrapib varied greatly, from no difference in AUC to a 3.8 increase when given with food. Food intake increased the AUC of an aqueous suspension by 18 times, while an oil solution showed a fivefold increase in AUC (84). Thus, the largest part of the food effect can be removed simply by administering the drug in solution in oil, while the last step to a complete elimination of the food effect for a specific drug using SMEDDS requires elaborate formulation work, employing many different lipid excipients.

Another approach to lipid-based drug delivery systems are lyophilized dry emulsion (LDE) tablets that reform an emulsion containing the drug in solution upon ingestion. A study in healthy volunteers showed that administration of griseofulvin in LDE resulted in no statistical differences between the fasted and the fed group. Further, the LDE seems to be independent of water intake (63).

The reduction of drug particle size can also lead to a reduced food effect because of a faster dissolution in the GI tract with less dependence on the solubilizing capacity of the GI fluids. Micronizing fenofibrate particles in aqueous media, in the presence of PL, resulted in submicron particles with their surface stabilized by PL. These particles had a faster dissolution, which in turn suppressed the food effect. The AUC of the plasma fenofibric acid curve was unchanged by food, while $C_{\max}$ increased just 1.4 times (85). A further reduction in the particle size of fenofibrate through nanosizing the drug was able to eliminate the food effect on $C_{\max}$ as well as AUC (86).

Dosage forms containing amorphous drug, like solid dispersions or solid solutions, have also shown reduced food effects, again due to the increased dissolution rate of the drug in its amorphous form. This has been shown for Kaletra[®], which contains both ritonavir and lopinavir as solid dispersions (87). Food effects that are not related to solubility or dissolution rate of the drug, but are mediated by food component effect on absorptive transporters, efflux transporters, or metabolism, are more difficult to address because of their physiological rather than physical source. The most logical way out of this paradox is to avoid intake of the particular food together with the drug.

In conclusion, formulation approaches to eliminate food effects primarily focus on dosing the drug in solution or in increasing the dissolution rate of the compound.

CONCLUSION

Intake of a meal induces many and various changes in the GI tract, which are to a high degree dependent on the composition and amount of food that has been ingested. Most changes have impact on drug bioavailability, primarily depending on the physicochemical characteristics of the drug. Further, the behavior of the GI tract in the fed state can also have impact on the transit and disintegration of dosage forms. Formulating to eliminate the food effect is a complicated endeavor, mainly focused on keeping the drug in solution or in an easily dissolved form.

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Oral Drug Absorption in Pediatric Populations

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INTRODUCTION

A paradigm shift is taking place from protecting children against clinical research to protecting them through research (1). This is based on the right of children to safer and more effective medicines, the overall increase in the role of medicines in disease management, and the better use of information pertaining to the physiology of children (1). It is recognized that there are challenges in optimizing the development, availability, and routine use of effective, safe, and affordable medicines for children (2). Initiatives such as the World Health Organization's (WHO) campaign "make medicines child size" in December 2007 and the International Alliance for Better Medicines for Children in 2006, and initiatives by the European Medicines Evaluation Agency (EMA) and the Food and Drug Administration (FDA) in the USA to regulate and give incentives to the pharmaceutical industry to conduct pediatric clinical trials for new investigational drugs have spurred a global drive to produce more and better medicines specifically for children. A better understanding of how children differ from adults with respect to drug absorption and use of this information to design more appropriate dosage forms for children are integral components of these initiatives.

The oral route of administration is commonly used for dosing medicinal products to pediatric patients. Adults can be considered a relatively homogenous population in terms of anatomical and physiological aspects of body organs in comparison to the striking changes that are seen throughout development (3,4). These changes can impart major differences in pharmacokinetic properties, such as absorption, distribution, metabolism, and excretion of the drug between children and adults, and may influence the efficacy, toxicity, and dosing regimens required in children (4–6). Two main facets of gastrointestinal (GI) development, that is, growth and maturation, affect the absorption of drugs from the pediatric GI tract. Growth refers to the addition of mass or volume and resulting changes in surface area to volume ratio, which in turn are related to total transport capacity. Maturation refers to the approach of GI tract physiology to that of adults, including parameters such as luminal and intestinal wall enzyme activity, gastric emptying (GE), intestinal motility, and composition and rate of secretions in the GI tract. This chapter will focus on reviewing literature relevant to the growth and maturation of the GI tract and their influence on drug absorption following oral administration to the pediatric population.

With respect to drug prescribing in children, the following age groups, related to developmental stages, have been defined (7):

- Preterm newborn infants, preterm neonates;
- Term newborn infants, term neonates (0–27 days);
- Infants and toddlers (1–23 months);
- Children (2–11 years); and
- Adolescents (12–16 or 12–18 years).

For the purposes of examining the effect of development on drug absorption, physiological parameters for some or all (where applicable) of these age groups will be presented. Also explored is how pediatric oral formulation development may be facilitated by this knowledge.

GASTROINTESTINAL TRACT DEVELOPMENT

pH

Despite a near neutral gastric pH (pH 6–8) at birth, which is related to the presence of amniotic fluid in the stomach (8), the resting pH of the neonatal stomach shortly after the initiation of feeding is similar to that in adults, with a value of approximately pH 2 (9). Hydrochloric acid secretion in neonates is lower than that in adults (10), resulting in a lower buffering capacity of the stomach, which in turn leads to a protracted high pH following feeding (9). This lower buffer capacity has been linked to an increased bioavailability of the acid-labile drug, penicillin G, in premature and term neonates less than two weeks old compared to infants and children (11). Furthermore, since unionized drug is better absorbed, a higher pH is expected to decrease the rate and/or extent of absorption of weak acids [e.g., phenytoin (12), rifampicin (13)], while weak bases such as atropine, caffeine, as well as other methylxanthines may be absorbed more readily from the stomach (14).

However, the primary site of absorption is the small intestine. While studies of small intestinal pH in neonates are not available, studies on the small intestinal pH from the age of six months in the fasted state is similar to that in adults (15,16).

Gastric Emptying

The time taken for the stomach to empty its contents into the small intestine is prolonged in neonates and infants in comparison to children and adults. The volume of stomach contents in the fasted state is approximately 3 mL for neonates and infants, approximately 10 mL for children, and 50 to 222 mL for adults (17,18). The volume can increase up to 50-fold after feeding. The volume of a meal, its osmotic pressure, and its composition of macronutrients have a major effect on the rate of GE. For instance, the type of fatty acids fed to infants affects the rate of GE; slower emptying is observed after feeding with long-chain fatty acids compared to medium-chain triglycerides (5,19). Both the type of food and the less pronounced gastric contractions in neonates and infants, with those in neonates less pronounced than those in infants (20), lead to prolonged GE in neonates and infants compared to children and adults.

In 2003, Van Den Driessche and Veereman-Wauters (21) presented a review of the literature on the age dependence of GE, and Table 1 summarizes

TABLE 1 The Age Dependence of GE Time as a Function of Diet Type and Measurement Method

Diet	Age [range] (SD)	Method	GE halftime (min) [range] (SD)	Reference
Liquid meal	28.9 wk [26–33]; PNA 19 days [6–37]	Scintigraphic method	60 [30–180]	Bode et al. (22)
	34 wk (1.5); PNA few days	¹³ C-octanoic breath test	50.3 (29.9)	Pozler et al. (23)
	13.4 wk (7)	Scintigraphic method	116.1 (72)	Vivatvakin and Buachum (24)
	4.7 mo [4–6]	Echography method	124 (9.7)	Garzi et al. (25) ^a
	9.2 mo (4.3)	Ultrasonographic method	74.7 (3.0)	Shaaban et al. (26) ^a
	Adults	¹³ C-octanoic breath test	35 [10–110]	Maes et al. (27)
	Adults	Scintigraphic method	20 [10–33]	Bouras et al. (28) ^a
Semisolid meal	9.2 mo (4.3)	Ultrasonographic method	101.8 (3.6)	Shaaban et al. (26) ^a
Solid meal	8–14 yr	Radiotransmitting capsule method	66 (mean residence time of capsule) ^b	Fallingborg et al. (16)
Solid meal (150 kcal)	5–10 yr	Scintigraphic method	107 [55–160]	Singh et al. (29)
Solid meal (150 kcal)	Adults	¹³ C-octanoic breath test	55 [35–130]	Maes et al. (27)
Solid meal (250 kcal)	Adults	¹³ C-octanoic breath test	94 [50–135]	Maes et al. (27)
Tablet	Adults	Scintigraphic method	62 (17)	Ofori-Kwakye et al. (30)

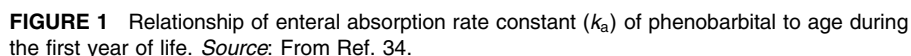
^aGE time values taken from the control/healthy/reference group used in the study.

^bNot significantly different to the mean residence time in the tested adults.

Abbreviation: PNA, postnatal age.

more recent literature that is not included in that review. For premature and term neonates, the halftime for GE is prolonged in comparison to adults (see Ref. 21 and Table 1) with the half-emptying time of milk declining with age. Although water is rapidly emptied, with a half-emptying time from just 7 minutes in term neonates to 15 minutes in infants aged 2 to 24 months (21), the relevant emptying time for drugs in neonates and infants, in whom there is almost a constant presence of food in the stomach, is expected to be that of milk and/or formula, which have GE half-lives of around 1 to 1.5 hours. The GE of solids and semisolids in children aged 4 to 15 years (21) and the mean residence time of a radio-transmitting device in the stomach of children aged 8 to 14 years (16) did not show differences to the corresponding values in adults.

Prolongation of GE in neonates and infants may have implications for the rate of absorption of drugs in cases where GE rate is the rate-limiting step to absorption. For example, slow GE is held responsible for the lower rate of absorption of acetaminophen (31,32) and busulfan (33) and is thought to be also at least partially responsible for slower absorption of D(+)-xylose, L(+)-arabinose, sulfonamides, phenobarbital, digoxin, β -methyl digoxin (34), nafcillin (35), ampicillin (35), riboflavin (36), and levetiracetam (37). On the other hand, calculated rates of absorption for some substances, such as digoxin and xylose, have demonstrated that although prolonged GE is present in neonates, it does not completely account for the delays seen in absorption of these compounds (34)



Small Intestinal Transit

Intestinal Permeability

Following oral administration of lactulose and mannitol or lactulose and L-rhamnose, intestinal permeability (P_{int}) ratios can be determined. These compounds move through the gut wall by different routes in newborns; lactulose by the paracellular pathway through the tight junctions, and

TABLE 2 The Age Dependence of SITT as a Function of Measurement Method

Age	Method	SITT (hr) [range] (SD)	Reference
28.9 wk [26–33]; PNA 19 days [6–37]	Scintigraphic method; Orocecal	3.1 [1.6–6.1]	Bode et al. (22)
2.3 mo [0–1 year]	Lactulose H ₂ breath test; Orocecal	1.6 [1.2–2.0]	Vreugdenhil et al. (40)
1.4–4.9 yr	Fructose H ₂ breath test; Orocecal	0.88 [0.5–2.0]	Hoekstra (41)
1–5 yr	Hydrogen breath test; Orocecal	1.5 (0.34)	Khin et al. (42)
7.3 yr [1–14]	Lactulose H ₂ breath test; Orocecal	1.4 [0.83–2.0]	Vreugdenhil et al. (40)
3–17 yr	Lactose-[¹³ C]ureide breath test; Orocecal	4.3 [2.8–6.5]	Van dan Dreissche et al. (43)
3–13 yr	Hydrogen breath test; Orocecal	3.4 (0.39)	Soares et al. (44) ^a
8–14 yr	Radiotransmitting capsule method; SITT	Same as adults	Fallingborg et al. (16)
Adults	Lactulose H ₂ breath test; Orocecal	1.2 [0.5–2.3]	Vreugdenhil et al. (40)
Adults	Scintigraphic method; SITT	Means 3.7, 4.0, 3.8, respectively [1.44–6.6]	Ofori-Kwakye et al. (30), Graff et al. (45), Bouras et al. (28) ^a

^aTransit time values taken from the control/healthy/reference group used in the study.

Abbreviations: SITT, small intestinal transit time; PNA, postnatal age.

mannitol and L-rhamnose by a transcellular pathway. Because they do not undergo any metabolic alteration and are excreted only by renal processes, the excretion ratio is a gauge of the predominant pathway of uptake in the gut. In the first few days of life uptake via the paracellular route is high, as evidenced by a high lactulose/mannitol excretion ratio of 1.6, which decreases to 0.6 over the first seven days of feeding (46). A decline in the importance of the paracellular route for uptake occurs even in preterm neonates shortly following the initiation of feeding (46,47), rapidly reaching ratios similar to those in infants and toddlers aged one month to three years (48) and in adults (49). The implications of high paracellular permeability may influence drug absorption for neonates who have not yet fed, although examples of the effect of age-dependent P_{int} on drug absorption are lacking.

Small Intestinal Surface Area

Intestinal surface area is a function of the radius and length of an intestinal segment as well as the amplification of this surface area because of folds, villi, and microvilli. Each intestinal segment has a unique continuum of villi that differs with respect to quantity and structure (50), and age influences these parameters. For instance, the villi structure of the jejunum in infants and children less than three years of age is observed to consist of single projections. Villi are leaf or finger shaped in children and adults over three years of age (50–52). Quantitatively, the median number of villi/intestinal area in infants and

children from 11 months to 3 years of age were 2.5 times lower than those for children aged 3 to 14 years (51). Both the structure and quantity of villi in younger children and infants suggest that intestinal surface area may be lower in these age groups, which in turn could impact oral drug absorption. Heimann (34) observed that, for some substances, GE and intestinal motility could not be wholly responsible for the age dependence of the rate of absorption, and it is probable that the age dependence of intestinal surface area is a contributor.

Luminal Composition in the Upper GI Tract

GI enzyme secretion and activity is different in the pediatric population. The secretion of pepsin (a gastric enzyme involved in the digestion/degradation of proteins and peptides) is increased threefold between week 35 of gestational age and term and a further fourfold increase is observed during the first two days after birth. Pepsin activity is relatively low in preterm neonates, whereas hydrochloric acid secretion is about the same as in term neonates (5,53). The secretion of pepsin gradually increases during the first months and is comparable with that of adults when expressed on a body-weight basis by the age of two years (pepsin activity: 125 U/mL gastric aspirate in infant compared to 600 U/mL gastric aspirate in adults) (53,54). The activity of gastric lipase (an enzyme involved in the digestion/degradation of triglycerides) was found to be 10 U/mL in gastric aspirates from infants compared to 5.7 U/mL in gastric aspirates from adults (54) and is more susceptible to dietary changes than pancreatic lipase (55).

A functional immaturity of the pancreatic exocrine secretion rate seems to exist even in the full-term neonate (56). Pancreatic enzymes' activity is related to diet; increased output of trypsin and amylase is observed after a high-protein diet or a diet containing starches, in preterm neonates, but a high-fat diet had no effect on lipase output (5). Pancreatic lipase activity in the preterm neonate (34–36 weeks' gestation) is only half of that in term neonates, and a 10-fold increase is observed between birth and nine months of age (5). Boehm et al. (57) reported that lipase activity in preprandially aspirated duodenal juice of preterm neonates of gestational ages 29 to 32 weeks and 33 to 36 weeks was 13.7 ± 7.9 and 15.9 ± 9.8 U/mL, respectively, and reached approximately 35% of the activity in infants and children (<6 years) after six weeks of postnatal life; trypsin activity in the same groups was 7.9 ± 4.7 and 8.5 ± 5.1 U/mL, respectively, and reached the value observed in infants and children (<6 years) within the first month of life. The low pancreatic lipase levels in preterm neonates are compensated for by lipolysis in the stomach by gastric lipase and by the intestinal hydrolysis of fat through the action of human milk bile salt-stimulated lipase (58). Therefore, despite the physiological pancreatic deficiencies, the term neonate absorbs over 85% of lipids in the maternal milk (58). The activity of amylase (an enzyme involved in the degradation of carbohydrates) is very low at birth and increases 200-fold by the age of nine months (5). Aminopeptidase and carboxypeptidase activities had a value of 0.072 U/mg protein (± 0.005) and 0.021 U/mg protein (± 0.005), respectively, in infants, children, and adolescents (59), indicating a lack of age dependency in these enzymes.

Luminal enzymes such as lactase that are involved in the digestion of milk carbohydrate have lower activity in the adult than the neonate (60,61). On the other hand, the intestinal brush border hydrolases such as maltase, sucrase, and isomaltase that are involved in digestion of carbohydrates of solid food are low at birth (62) and increase in activity as adulthood is approached (63). The activity

of intestinal aryl hydrocarbon hydrolase also increases with age. By contrast, the activity of other hydrolases such as epoxide hydrolase and glutathione peroxidase is independent of age (64). The differences in the activity of the gastric, pancreatic, and luminal/brush border enzymes in pediatric populations may affect the performance of a drug or the bioavailability of an oral formulation, especially the absorption from lipid formulations (6).

Bile is a complex fluid, containing water, electrolytes and bile acids, phospholipids, cholesterol, and bilirubin, which aids the solubilization of the poorly water-soluble products of lipid digestion, for example, fatty acids, as well as enhancing the solubility of poorly water-soluble drugs (65). Primary bile acids (cholic acid and chenodeoxycholic acid) are synthesized in the liver and conjugated to glycine or taurine prior to secretion into bile. Secondary bile acids—deoxycholic and lithocholic acid—are formed by dehydroxylation of the primary acids by bacterial enzyme systems (5). The cholic acid:chenodeoxycholic acid ratio is high in neonates (2.5:1 in neonates compared to 1.2:1 observed in adults) and decreases with postnatal age (5,66). Near-term bile flow is low compared to adult levels (5,17). In neonates and infants, the bile is composed of only primary bile acids; in neonates, the bile acids are preferentially conjugated with taurine but the glycine:taurine ratio increases with postnatal age (5,17).

In neonates, intraluminal bile acid concentrations of 1 to 2 mmol/L have been found after meal stimulation, with little variation throughout the day (17,65–67). The ability of the neonatal gallbladder to concentrate bile acids appears to be less developed than that of adults, in whom intraluminal bile salt concentrations postprandially often reach 10 to 15 mM (65). The mean gallbladder volume prior to meal intake in children is in the range of 13.8 to 18.8 mL compared to an average of 27.2 mL observed in adults (54,68,69). Active reabsorption of bile acids is observed, starting at about eight months after birth (5). The lower bile acid pool size and the immature mechanisms for intestinal reabsorption in infants lead to remarkably high levels of bile acids in the serum—a condition referred to as *physiologic cholestasis* or *physiologic hypercholanemia* of infancy (65). As much as 50% to 60% of the bile acids may be reabsorbed in the jejunum and colon of infants, whereas in children and adults the primary site of reabsorption is the distal ileum (5,67). The age-related differences in bile acid transformation and enterohepatic bile acid circulation may be due to the absence of an appropriate microbial flora necessary for these processes (5). At birth the intestine is virtually sterile, but then a rapid colonization occurs with a flora that differs between breast-fed and formula-fed infants, with further changes occurring during development (14).

Bile acts, to some extent, as a surfactant and is involved in the digestion and absorption of lipids. Inefficient intestinal fat digestion in neonates can occur as a consequence of low bile excretion (70). Reduced absorption of fat-soluble vitamins—vitamin D and vitamin E—in neonates can probably be attributed to the inadequate bile salt pool and low intraluminal levels, despite the higher circulating systemic concentrations (6,14,70,71). Similarly, lipophilic agents that require solubilization in the intestine may display capacity-limited absorption in the neonate.

Brush Border and Hepatic Enzymes and Transporters

The maturation of transporters/enzymes involved in drug absorption/first-pass metabolism parallels the structural or functional maturation of the digestive

system (72). Maturation patterns of transmembrane proteins such as intestinal metabolizing enzymes and intestinal transport proteins remain largely unknown, despite the appearance of a handful of related papers and a recent review on the topic by Johnson et al. (73). Johnson et al. (74) assayed CYP3A4 activity in duodenal biopsies from 74 histologically normal pediatric samples. Activity was absent in fetal samples but increased significantly from the ages of 2 weeks to 17 years. In another study (75), CYP3A4 mRNA levels in small intestinal samples were low in fetal samples, increased to twofold in neonates less than one month old and sixfold in children and adults more than 15 years old, although differences were not statistically significant ($p > 0.05$). In a third study, duodenal biopsies from infants and children aged 1 month to 17 years demonstrated a significant ($p < 0.001$) decline of CYP3A4 and a nonsignificant decline in CYP3A5 mRNA levels with age (76). Results in the literature to date are not conclusive.

In studies of the P-glycoprotein (P-gp/ABCB1), multidrug resistance (MRP1/ABCC1), and breast cancer resistance (BCRP/ABCG2) transporters that assist in transmembrane flow of xenobiotic substrates, lower expression in the central nervous system is observed in neonates than in adults (77). This trend is mirrored in the small intestine. Miki et al. (75) observed lower MDR1 (membrane-associated protein encoding for P-gp) expression in neonatal samples compared to those of children and adults (15–38 years). However, no age dependence of P-glycoprotein mRNA levels was found in small intestinal samples of infants and children of ages 1 month to 17 years (76). Cyclosporine is both a CYP3A4 and P-glycoprotein substrate and interactions with both of these contribute to its low oral bioavailability. In a study with 20 infants and children aged 1 to 17 years, oral bioavailability was observed to be age independent (78), suggesting that intestinal clearance (coordination of CYP3A4 with P-glycoprotein transporters) was operating with reasonable efficiency in children.

First-pass effects are influenced by both intestinal and hepatic enzymes and transporters. Many hepatic enzymes have reduced activity in children in comparison to the adult liver, and numerous reviews exist on the subject (79,80). The implication of reduced hepatic activity is an increased bioavailability of orally administered drugs, as has been demonstrated for midazolam (81) and zidovudine (82).

The magnitude of the age dependence of bioavailability for any given drug is partially dependent on the proportion of the drug metabolized by presystemic enzymes. For example, a drug such as acyclovir, which is almost exclusively eliminated renally, has a bioavailability in neonates equal to that in adults (83), whereas the previous examples of midazolam and zidovudine, both of which are extensively hepatically metabolized, show age dependence in oral bioavailability. Hepatic transporters may also play a supporting role with respect to the hepatic extraction of drugs. In rats, mRNA expression for transporters mediating hepatic uptake of substrates (e.g., organic anion transporting polypeptides, Oatp1a4) is low at birth and increases during development (84). On the other hand, mRNA expression of transporters mediating transfer of molecules from the hepatocyte into the bloodstream (e.g., MRP1) is highest at birth and gradually decreases with increasing age (84). The human database shows that MDR1 expression is not present in fetal liver but is expressed at one month of age (85). Comparisons with expression levels in adults were not made in that study.

DRUG ABSORPTION IN THE PEDIATRIC POPULATION

Table 3 presents literature regarding the age dependence of absorption for various compounds on the basis of *in vivo* pharmacokinetic studies. Interpretation of studies for the parameters describing absorption is complicated by sparse sampling, especially in the neonatal age groups. As a result, apparent $t_{\max}$ is not a good indicator of the age dependence of absorption, since often only one or two blood samples are taken during the absorptive phase. There are some exceptions to this generalization, for example, Kearns et al. (92). For the most part, absorption in neonates and infants is slower than that of children and adults. Additionally, the age dependence of bioavailability is drug dependent, as highlighted in Table 3.

Practical issues may have a significant effect on the absorption of drugs in children and should be duly noted when interpreting the pharmacokinetic studies listed in Table 3. For example, infants need frequent feeding and, similarly, medication in children is often combined with food or fruit juice. Co-administration with feeds/meals could have a detrimental influence on efficacy and safety for a variety of reasons including effects on absorption, bioavailability (94), and through inaccurate dosing (39). The almost continuous presence of milk in the stomach in neonates and infants may limit the absorption of compounds, which are lipid soluble (due to lack of lipases) or bind to protein (5). It is worth noting that very few well-controlled stability studies have been published on compatibility issues between ground or suspended solid dosage forms and food/beverages (95–98). Further, in some cases children being administered medications may have a disease that alters oral drug absorption, such as celiac disease or cystic fibrosis. In any case, it is clear that further studies focused on determining the age dependence of drug absorption in certain disease states are required (73).

ORAL FORMULATION DEVELOPMENT FOR THE PEDIATRIC PATIENT

The different physiological development stages of the various pediatric subgroups pose challenges to drug development. The knowledge of absorption characteristics in the pediatric population, as described earlier, can be used to facilitate oral formulation development for specific pediatric subpopulations. For example, the difference in the volume of GI fluids in the pediatric population compared to the adult population could have a significant effect on the dissolution rate of an oral formulation. The ability of the pediatric population to absorb drugs is especially of great importance for the development of certain dosage forms for which the physiological factors are more important than technological factors. For example, drug absorption after an enteric-coated formulation will be highly dependent on GE rate. Protocols for studies evaluating food effects on formulation and for food-drug interactions also need to be specially designed for the pediatric population.

A variety of different oral dosage forms are available for each pediatric age group. There is often no single formulation that is ideal for pediatric patients of all ages, and a range of dosage forms is usually preferred during formulation development. Important considerations for pediatric formulation development and the selection of an appropriate formulation for each age group are: (i) sufficient bioavailability, (ii) minimal dosage frequency, (iii) minimal impact on the

TABLE 3 Age Dependence of Oral Drug Absorption

Parameter	Compound	Mean age [range] (SD)	Change in parameter	Remarks from reference	Reference
Bioavailability (<i>F</i>)	Penicillin G	■ Premature neonates (average PNA 23 days)	Increased <i>F</i> in premature and term neonates compared to older children	Owing to lower gastric acid production and reduced acid hydrolysis of penicillin	Huang and High (11)
		■ Full-term neonates (average PNA 3 days)			
		■ Older infants and children (PNA > 2 wk–13 yr) (<i>n</i> = 8–18 per group)			
<i>F</i>	Midazolam	■ GA 26–31 wk, PNA 3–13 days (<i>n</i> = 15)	Absolute <i>F</i> higher (49%) than historical adults (24–38%)	Because of reduced intestinal and hepatic CYP3A activity	Hoppu et al. (78)
<i>F</i>	Zidovudine	■ Neonates < 14 days	Absolute <i>F</i> higher (89%) in younger group compared to older (61%) group	Because of reduced first-pass	de Wildt et al. (81)
		■ >14 days–3 mo (<i>n</i> = 32)			
<i>F</i>	Phenytoin	■ 11 days [1–123] (<i>n</i> = 83)	<i>F</i> lower in neonates (75%) compared to literature-based adults (100%)		Al Za'abi et al. (12)
<i>F</i>	Cyclosporine	■ 3.4 years [1.1–16.8] (<i>n</i> = 20)	<i>F</i> not age dependent and similar to adult values from the literature	Low <i>F</i> due to poor absorption and prehepatic metabolism	Hoppu et al. (78)
<i>F</i>	Pleconaril	■ 7–32 days (<i>n</i> = 16)	Suggestive of increased <i>F</i> in older children and adults compared with neonates		Keams et al. (6)
<i>F</i> , first-order absorption rate constant (<i>k_a</i>)	D(+)-Xylose, L(+)-arabinose, sulfonamides, phenobarbital, digoxin, β-methyldigoxin	■ [Days to 14 years] (<i>n</i> = 580)	No age dependence of <i>F</i> , increased <i>k_a</i> with age	Because of more than prolonged GE and increased intestinal motility	Heimann et al. (34)

(Continued)

TABLE 3 Age Dependence of Oral Drug Absorption (*Continued*)

Parameter	Compound	Mean age [range] (SD)	Change in parameter	Remarks from reference	Reference
F, k_a	Riboflavin	■ Term neonates (PNA 5-6 days) ($n = 2$)	No age dependence of F , increased k_a with age	Because of slow intestinal motility and a prolonged transit time through the gut in infants	Jusko et al. (36)
		■ Older infants (e.g., 10 mo)			
k_a	Acetaminophen	■ Term neonates (PNA 49-74 hr) ($n = 12$)	Slower absorption than adults	Owing to prolonged GE	Levy et al. (31)
Urinary excretion of reduced digoxin metabolites	Digoxin	■ Infants (3 days-8 mo) ($n = 36$)	No urinary excretion of reduced digoxin metabolites until the age of 8.5 mo	Reduced capacity of anaerobic bacteria in enteric flora of infants, potentially leading to an increase in digoxin bioavailability in infants	Linday et al. (86)
		■ Children (8 mo-21 yr) ($n = 51$)			
k_a	Levetiracetam	■ 9.8 yr [0.2-18] ($n = 228$)	Increased k_a with age	Plateau at 10 yr of age	Toublanc et al. (37)
Half-life of absorption ($t_{1/2abs}$)	Acetaminophen	■ Under 3 mo	$t_{1/2abs}$ In children < 3 mo was 3.68 times greater than children over 3 mo		Anderson et al. (32)
		■ Over 3 mo			
k_a	Busulfan	■ [1.5-6 yr] ■ [13-48 yr]	Young children showed a slower (not significant) rate of absorption than the older group	Suggested to be due to prolonged GE	Hassan et al. (33)
k_a	Busulfan	■ 9.9 years [0.4-18] ($n = 48$)	No age dependence of k_a	Widely varying busulfan oral formulations used	Schiltmeyer et al. (87)
k_a	Cefibuten	■ 7.8 years [1.2-16.4] ($n = 49$)	No age dependence of k_a		Keams et al. (88)

k_a , time of maximum plasma concentration	■	Term neonates [PNA 24–48 hr]	Lower k_a than in adults, greater t_{max} than adults	Adults given 500 mg capsule, neonates given 10 mg/kg as drops	Silverio and Poole (35)
t_{max} , F	■	6 mo–16 yr ($n = 85$)	No age dependence of t_{max} or F		Chien et al. (89)
t_{max} , F	■	[2–17 yr] ($n = 45$)	No age dependence of t_{max} or F		Sokal et al. (90)
t_{max}	■	10.8 yr [5–16] ($n = 24$)	No age dependence of t_{max}		Keams et al. (91)
Maximum plasma concentration (C_{max}), t_{max}	■	30.9 days (18.1) ($n = 17$)	t_{max} Decreased in oldest group (2.2 ± 1.1 hr)	Associated with developmental differences in GI motility	Keams et al. (92)
	■	40.8 days (21.0) ($n = 13$)	compared to two younger groups (4.4 ± 3.3 hr), C_{max} increased in oldest group		
	■	77.2 days (20.3) ($n = 5$)	compared to two youngest groups		
t_{max}	■	[1–16 yr] ($n = 26$)	No age dependence of t_{max}		Blumer et al. (93)
Phenytoin	■	Infants	Age-dependent absorption rate and extent influence of the food upon the absorption pattern		Albani et al. (94)
	■	Children			
	■	Adolescents			

Abbreviations: PNA, postnatal age; GA, gestational age; t_{max} , time of maximum plasma concentration; C_{max} , maximum plasma concentration; k_a , first-order rate of absorption; $t_{1/2abs}$, half-life of absorption; GE, gastric emptying; F , bioavailability.

life style of the child, (iv) a minimum of excipients in the formulation, (v) nontoxic excipients, (vi) convenient and reliable administration, (vii) stability, (viii) ease of the production process, and (ix) cost of the formulation (4,99).

Limited knowledge is available on the acceptability of different dosage forms, administration volumes, size of unit dosage, taste, and the acceptability and safety of excipients in relation to the age and developmental status of the child. The acceptance of certain dosage forms depends on several factors (i.e., child's mood, illness, cultural habits). The EMEA (7) has proposed a rough guide to indicate preferred formulations as a function of the age, on the basis of a matrix that combines different age groups and conventional dosage forms. It is important to note that this guide reflects only general aspects of acceptability of various dosage forms—as it is not an in-depth, evidence-based guide, but rather based on a questionnaire for hospital pediatricians, pharmaceutical scientists, and parents. This is a field where much more research is needed, so the guide should not be taken as a strict recommendation for the development of a specific dosage form for a given age group.

Extemporaneous products are widely used in pediatrics but the issues related to dosing accuracy, unknown stability, unknown bioavailability, and use of potentially toxic excipients create a great concern for their use (100). Currently, a wide variety of dosage forms are available for the pediatric population: solid dosage forms [powders; granules; crushable, granulate, or dispersible tablets; scored tablets; chewable tablets; orodispersible tablets; capsules and innovative delivery systems designed to reduce dose frequency (7,99,101)] and liquid formulations [aqueous solutions, suspensions, emulsions, and syrups (7,100,101)].

There is an increased interest in formulation development for the pediatric population to deal with the problems of extemporaneous administration and/or to improve the formulations available. The process involved in pediatric formulation development is different from that in adults (Fig. 2) (102). At the early stages of development, only very limited data are available to guide formulation

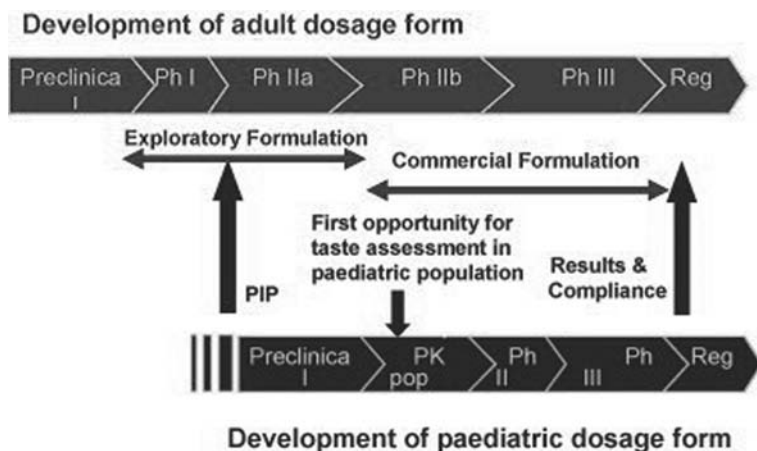


FIGURE 2 Dosage form development in adults and in children within the drug development framework. *Abbreviations:* PIP, pediatric investigation plan; Ph, Phase. *Source:* From Ref. 102.

development. The need for better formulations for children and the development of new formulations for oral administration, such as controlled release tablets, (mini) tablets, fixed dose combination tablets, and innovative drug delivery systems have been identified (99–103). For example, controlled release dosage forms have the potential to extend the period of time between dosing, reduce the number of doses required per day, and enhance patient compliance and patient/clinician convenience. This is particularly relevant for pediatric patients suffering with chronic conditions, which usually require regular dosing by the patient, parent, or teacher during the day (104). A once-per-day oral sustained-release theophylline formulation in children older than eight years of age showed little variation in absorption (105), whereas, in another study, inconsistent absorption was noted for some children aged 4 to 17 years (106). An understanding of the physiological changes associated with development is important for the development of safe and effective formulations for the pediatric population.

CONCLUSIONS

Until recently, most drugs prescribed to children have not had pediatric labeling and/or the clinical research to support labeling. Because of current regulations, pediatric clinical trials for new investigational drugs are becoming more frequent. Study design (i.e., definition of initial dose and optimization of sampling) and formulation development will benefit from a clear understanding of how age-dependent physiological factors and practical issues surrounding drug administration to children influence the absorption and bioavailability of drugs.

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INTRODUCTION

Most of the data about bioavailability of orally administered drugs are obtained in healthy individuals. However, many drugs are actually administered to patients in whom the underlying disease might affect either their absorption or their overall bioavailability. This is particularly true for patients suffering from diseases of intestinal absorption and digestion. In spite of a great potential importance for both clinicians and pharmacists, only a few reviews on this topic have appeared in the literature (1,2). Therefore, the aim of this chapter is to point out some gastrointestinal and systemic diseases that may affect drug absorption in humans.

Drug absorption from the gastrointestinal tract is influenced by the following factors:

1. Gastric and intestinal motility
2. Physicochemical properties of the environment in the gastrointestinal (GI) lumen
3. Mechanisms of and surface area available for absorption

Although the motility might influence the completeness of intestinal absorption, the intestinal environment can, in addition, dictate the physicochemical behavior of orally administered drugs. Gastrointestinal diseases, systemic diseases with intestinal involvement, and the consequences of gastrointestinal surgery may modify any or all of these factors. Subsequently, changes in the therapeutic effects of drugs administered by the oral route may occur. Bioavailability of orally administered drugs in patients with gastrointestinal disease can deviate greatly from that in healthy subjects, depending on the type and severity of the disease and complexities of the pathology involved. Consequently, both clinicians and pharmacists should be aware that in patients with gastrointestinal diseases, the possibility of altered bioavailability exists, and anticipate any monitoring of, or alterations in, therapy that are necessary.

Because gastrointestinal transit is discussed in detail elsewhere (see chap. 3), this review will be restricted to diseases of intestinal absorption and digestion and to literature related to their influence on drug absorption and bioavailability in humans.

INTESTINAL MALABSORPTION AND MALDIGESTION

In the last decades there has been an increase in our knowledge on the mechanisms and underlying diseases leading to intestinal malabsorption (3). A better understanding of the pathophysiology of fat digestion and absorption, definition of isolated defects of transport systems located at the apical membrane of the small-intestinal epithelial cells, mechanisms of bile acid absorption in health

and disease, and access to the intestinal lumen by means of specific laboratory tests are among the milestones that have been achieved in the investigation of pathophysiological mechanisms and consequences of intestinal malabsorption and maldigestion.

Malabsorption

Malabsorption is defined as impaired absorption of nutrients, occurring when there is either

1. a defect in membrane transport systems of the small-intestinal epithelium, without morphological changes (primary malabsorption) or
2. a defect in the epithelial absorptive surface, with concomitant morphological changes of the mucosa (secondary malabsorption).

In addition, there is a difference between global and partial (isolated) malabsorption syndrome (Table 1).

The major pathophysiological mechanisms in malabsorption are as follows:

Defects of the luminal phase of digestion

Defects of the mucosal phase of terminal digestion and absorption

Defects of the transport phase (Table 2)

Fat Malabsorption

Fat malabsorption may affect absorption of lipophilic drugs and drug formulations and, as such, deserves particular attention. The basic mechanisms of fat digestion and absorption are now well understood. By the action of pancreatic lipase, dietary triglycerides are degraded to monoglycerides and fatty

TABLE 1 Global and Partial/Isolated Malabsorption Syndromes

Global malabsorption syndromes

Small-intestinal disease with diffuse mucosal involvement

Celiac disease

Autoimmune enteropathy

Tropical sprue

Reduced absorptive surface

Short-bowel syndrome

Partial/Isolated malabsorption syndromes

Carbohydrate intolerance

Bile acid malabsorption

Bacterial overgrowth

Protein-losing enteropathy

Maldigestion in

Exocrine pancreatic insufficiency

Cholestasis

Bacterial overgrowth

Bile acid malabsorption

Gastrinoma

Small-bowel resection

Radiation enteritis

Intestinal lymphatic obstruction

TABLE 2 Pathophysiology of Maldigestion and Malabsorption

Luminal phase	
	Reduced nutrient availability
	Cofactor deficiency (pernicious anemia, gastric resection)
	Increased nutrient consumption (bacterial overgrowth)
	Impaired fat solubilization
	Reduced bile salt synthesis (hepatocellular disease)
	Impaired bile salt secretion (cholestasis)
	Bile salt inactivation (bacterial overgrowth)
	Impaired cholecystokinin release (mucosal disease such as celiac disease)
	Increased bile salt loss (terminal ileal disease or resection)
	Defective nutrient hydrolysis
	Lipase inactivation (Zollinger–Ellison syndrome)
	Enzyme insufficiency (pancreatic insufficiency)
	Improper mixing or intestinal hurry (resection, bypass, hyperthyroidism)
Mucosal phase	
	Extensive mucosal loss (resection, infarction)
	Diffuse mucosal disease (celiac disease, autoimmune enteropathy, tropical sprue, Crohn's disease, infections, drugs)
	Enterocyte defects
	Microvillus inclusion disease
	Tufting enteropathy
	Brush-border hydrolase deficiency (lactase deficiency)
	Transport defects (glucose-galactose malabsorption, Hartnup's disease)
	Epithelial processing (abetalipoproteinemia)
Transport phase	
	Vascular (vasculitis)
	Lymphatic (Whipple's disease, intestinal lymphangiectasia, radiation enteritis, tumor invasion)

acids. Bile salts in the jejunal lumen are responsible for micelle formation and subsequent solubilization of monoglycerides and fatty acids, allowing them to penetrate the intestinal mucosa. Triglycerides are resynthesized in the mucosa and, in the form of chylomicrons, are transported first to the intestinal lymphatics and subsequently into the general circulation.

Disturbances of each particular phase of dietary fat degradation, solubilization, and delivery into the systemic circulation may lead to fat malabsorption and steatorrhea. Impaired production or activity of pancreatic lipase (exocrine pancreatic insufficiency), disorders of bile acid metabolism (obstructive jaundice, bile acid malabsorption, and bacterial overgrowth), decreased absorptive surface area (resection, inflammation, atrophy), or abnormalities in lymphatic flow in the gut (Table 2) are among the potential causes of fat malabsorption. *Steatorrhea* (defined as stool fat > 7 g/day) leads to enteral loss of dietary fat, lipid-soluble vitamins, and calcium, as well as to an increased oxalate absorption with resultant "enteric" hyperoxaluria. Disturbances of bile acid metabolism may also cause steatorrhea and malabsorption by the impairment of the micellar phase of fat digestion in the small-intestinal lumen. An excess enteral loss of bile acids can be compensated by an increase of bile acid synthesis in the liver. This, however, often results in watery diarrhea owing to the impairment of water reabsorption in the colon by bile acids. If intestinal bile acid loss is higher than the synthetic capacity of the liver (e.g., in ileal resection or short-bowel syndrome), bile acid concentration in the intestinal lumen will be insufficient to induce micelle formation, resulting in decompensated bile acid malabsorption

and associated diarrhea and steatorrhea. Small-intestinal bacterial overgrowth is a consequence of morphological changes of the intestine (diverticula, fistulas, strictures, or stenosis) as well as motility disorders (diabetic gastroenteropathy and scleroderma). When deconjugated and dehydroxylated by the intestinal bacteria, bile acids exert a toxic effect on the colonic mucosa, again leading to a watery diarrhea. Because of the bacterial activity, the concentration of conjugated bile acids will be increased, resulting in malabsorption of fat and fat-soluble vitamins (A, D, E, and K). Bacterial overgrowth in the gut also leads to fermentation of carbohydrates in the intestinal lumen.

Carbohydrate Malabsorption

Starch, sucrose, and lactose are the most abundant digestible carbohydrates in the intestinal lumen. On the other hand, many of the polysaccharides originating from plants cannot be digested in the lumen. Impaired absorption of normally digestible carbohydrates may occur because of a lack in pancreatic α -amylase, defects in disaccharidase activity in the small-intestinal epithelium, or reduced absorptive intestinal surface. In primary carbohydrate malabsorption, single functional elements of carbohydrate digestion or absorption are missing (lactase, sucrase, glucose carrier) without apparent morphological changes. A generalized reduction of the intestinal absorptive surface can also lead to an impaired digestive and absorptive capacity in the gut (e.g., villus atrophy in celiac disease), resulting in secondary carbohydrate malabsorption (4).

Carbohydrates that are not digested and absorbed in the small intestine undergo bacterial degradation in the colon. The terminal phase of bacterial carbohydrate degradation is fermentation, resulting in formation of short-chain fatty acids (butyrate, propionate, acetate, lactate), as well as CO_2 , H_2 , and CH_4 . Short-chain fatty acids can be further utilized by the body through efficient reabsorption in the colon. Bacterial fermentation of carbohydrates secondary to malabsorption results in acidic stools, abdominal distension, meteorism, and flatulence. Similarly, dietary fibers can be degraded by bacterial enzyme activity in the colon; the extent of their degradation determines their effect on stool volume. Thus, poorly degradable fibers increase stool volume and regulate bowel movements, and are, therefore, useful in the treatment of constipation.

Protein Malabsorption

Impaired digestion and absorption of dietary protein occurs when pancreatic protease secretion or activity is impaired (exocrine pancreatic insufficiency), in rare isolated absorption defects (e.g., Hartnup's disease), and in generalized reduction of the intestinal absorptive surface (e.g., celiac disease). Of particular clinical importance is protein-losing enteropathy, in which plasma protein is excreted into the intestinal lumen, resulting in development of hypoalbuminemia and edema.

Maldigestion

Maldigestion is a consequence of impaired digestion of nutrients within the intestinal lumen, or at the terminal digestive site of the brush-border membrane of mucosal epithelial cells. It can occur because of congenital or acquired disease in which pancreatic enzyme activity, bile acid concentration, or small-intestinal mucosal enzymes are decreased or absent.

The pathophysiological possibilities leading to malabsorption-maldigestion syndrome are listed in Table 3. If there is impaired digestion, pancreatic insufficiency is the most frequent cause. The insufficiency may be due to chronic pancreatitis, pancreatic surgery, cystic fibrosis, pancreatic carcinoma, Zollinger–Ellison syndrome, (rare) congenital lipase deficiency, or postoperative postprandial pancreaticobiliary asynchrony (5). Cystic fibrosis, usually diagnosed in

TABLE 3 Diseases Resulting in Maldigestion and Malabsorption

Maldigestion caused by deficiency or inactivation of pancreatic enzymes
Chronic pancreatitis
Surgical resection of the pancreas
Pancreatic cancer
Cystic fibrosis
Zollinger–Ellison syndrome
Maldigestion caused by impaired luminal bile acid concentration
Obstructive jaundice
Intrahepatic cholestasis
Primary biliary cirrhosis
Primary sclerosing cholangitis
Small-intestinal bacterial overgrowth (blind loop syndrome, fistulas, strictures, diverticula, afferent loop syndrome, motility disorders in scleroderma, and diabetic gastroenteropathy)
Heal resection (decompensated bile acid loss)
Crohn's disease of the ileum
Maldigestion/malabsorption caused by small-intestinal diseases
Primary malabsorption: congenital diseases with selective defect of single functions of epithelial cells (disorders of the brush-border membrane)
Lactose intolerance
Sucrose-isomaltose intolerance
Trehalose intolerance
Enterokinase deficiency
Glucose-galactose intolerance
Cystinuria
Secondary malabsorption: acquired small-intestinal diseases
Celiac disease
Tropical sprue
Whipple's disease
Primary intestinal lymphoma
Hypogammaglobulinemia
Selective IgA deficiency
Eosinophilic gastroenteritis
Amyloidosis
Parasitoses (giardiasis, strongyloidosis, ascariidosis, ancylostomiasis)
HIV enteropathy with wasting syndrome
Tuberculosis
Lymphogranulomatosis
Kwashiorkor
Short-bowel syndrome
Intestinal ischemia
Radiation enteritis
Various disorders of digestion and absorption
Postgastrectomy syndrome
Postvagotomy syndrome
Diabetic gastroenteropathy
Endocrinopathies (hyper- and hypothyroidism, hyper- and hypoparathyroidism, Addison's disease, medullary carcinoma of the thyroid)
Glucagonoma, gastrinoma, VIPoma
Scleroderma

(Continued)

TABLE 3 Diseases Resulting in Maldigestion and Malabsorption (*Continued*)

Drug-induced malabsorption

Cholestyramine
Laxatives
Colchicin
Antineoplastic drugs
Neomycin
<i>p</i> -Aminosalicilic acid (PAS)
Biguanides
Lactulose, sorbitol, fructose
Nonsteroidal anti-inflammatory drugs (NSAID)
Alcohol

childhood, leads to exocrine pancreatic insufficiency in adults. Chronic diarrhea with accompanying steatorrhea can also occur in gastrinoma (Zollinger–Ellison syndrome). In this case, the high volumes of gastric juice entering the small intestine prevent the critical bile acid concentration necessary for the formation of micelles during fat digestion from being reached and also inactivate pancreatic lipase owing to the low pH.

Postoperative syndromes (gastric resection by Billroth II operation, vagotomy, Whipple's operation) not only lead to motility disorders, but can also, in spite of preserved function of the exocrine pancreas, lead to a disturbed digestion. The mechanism responsible for this disorder is the rapid gastric emptying that induces impaired or decreased hormonal stimulation of the exocrine pancreas (postprandial pancreaticobiliary asynchrony) (6).

Maldigestion can also develop when critical micellar concentration of bile acids is insufficient to contribute to fat digestion (intraluminal impairment of bile acids) under the following conditions:

1. If there is an impaired secretion of bile acids into the lumen (obstructive jaundice, intrahepatic cholestasis, primary biliary cirrhosis)
2. If there is an extensive bile acid loss from the lumen, higher than the synthetic capacity in the liver
3. If bile acids are deconjugated in the intestinal lumen owing to bacterial overgrowth syndrome

An increased enteral bile acid loss occurs most frequently in Crohn's disease with ileal involvement and after surgical resection of the ileum. If less than 1 m of ileum is removed or functionally impaired, a compensated chologenic diarrhea occurs, and it can be efficiently treated with ion-exchangers (cholestyramine, cholestipol). Diarrhea is watery and occurs because of the laxative effect of bile acids on the large bowel mucosa. If enteral bile acid loss exceeds the maximal synthetic capacity of the liver (e.g., ileal resection of more than 1 m), an impairment of the ability to reach the critical bile acid micellar concentration occurs, with consequent fat maldigestion and steatorrhea. This decompensated chologenic diarrhea will become even worse if treated with anion-exchange resins.

Ingestion of a nonabsorbable artificial sweetener, sorbitol, can lead to an osmotic diarrhea ("chewing gum diarrhea"). As little as 5 g sorbitol can induce intestinal symptoms, and 10 g leads to meteorism, flatulence, and diarrhea. The

symptoms can be worsened by the addition of fructose, which is also poorly absorbed in the gut.

GASTROINTESTINAL DISEASES THAT CAN INFLUENCE DRUG ABSORPTION

Drug absorption in patients with gastrointestinal disorders is influenced by changes in gastric and intestinal motility, changes in the surface area available for drug absorption, and altered physical and chemical properties of the intestinal luminal content. These properties are usually changed in combination, the degree of each being dependent on the duration and severity of the disease.

Crohn's Disease

The incidence of Crohn's disease has been increasing over the years; in the United States, the total population with this disease is estimated to be 200,000 to 400,000, with 15,000 to 30,000 new cases occurring each year. The etiology of Crohn's disease remains unknown, although various infectious agents, immunological causes, and familial clustering are thought to contribute to its development. Although Crohn's disease may be distributed along the entire intestine, it most commonly involves the ileocolic region. More than one area of the gut can be affected, while the bowel in between appears normal, giving rise to so-called skip areas. Typical manifestations are a thickening of the intestinal wall, mucosal fissures, fistulas, inflammatory masses, and benign strictures. The resulting clinical findings include diarrhea, abdominal pain, fever, and weight loss.

Because of its highly variable clinical presentation, Crohn's disease may lead to impaired drug absorption by any of the three mechanisms of malabsorption: the thickened bowel wall and strictures may significantly alter the bowel motility; mucosal lesions may lead to changes in intestinal permeability; and involvement of specific intestinal areas (e.g., terminal ileum) may cause bile acid malabsorption and subsequent fat maldigestion. Although nowadays this disorder can be treated efficiently with anti-inflammatory agents (e.g., 5-aminosalicylic acid and its derivatives, and corticosteroids), anatomical changes of the intestine may well result in altered drug absorption, even in patients in remission. For this reason, particular emphasis should be given to drug dosage and formulation in patients with Crohn's disease.

Celiac Disease

Celiac disease is characterized by atrophy of the small-intestinal mucosa, with subsequent impairment of absorption of all nutrients, including fat. It is caused by hypersensitivity to a protein present in wheat: gluten. Elimination of wheat (gluten-free diet) results in the normalization of small-intestinal morphology and restored absorptive function.

AIDS Enteropathy

As a part of acquired immunodeficiency syndrome (AIDS), diarrhea and a general wasting syndrome frequently occur (7). This is not always due to accompanying infections, the AIDS virus itself can damage the intestinal mucosa. Patients with AIDS enteropathy have an abnormal carbohydrate

malabsorption, bacterial overgrowth of the intestine, often also some disaccharidase deficiency, bile acid malabsorption, and steatorrhea (8). Reduced total drug exposure is related to malabsorption in persons with human immunodeficiency virus (HIV) infection or AIDS (9).

Furthermore, intestinal permeability in patients with AIDS enteropathy is increased, in a way similar to celiac disease. The duodenum of HIV-infected patients with diarrhea showed an impaired epithelial barrier function, which was thought to contribute to diarrhea by a leak-flux mechanism (10). Despite the increase in permeability, some protease inhibitors used in the treatment of patients with HIV infection (e.g., saquinavir) have kinetic profiles characterized by reduced absorption and a high first-pass effect, resulting in poor bioavailability. Administration with food leads to an improvement in absorption in the case of saquinavir. Further pathophysiological factors, such as achlorhydria, malabsorption, and hepatic dysfunction, may also influence the bioavailability of the protease inhibitors in patients with HIV disease (11).

Small-Intestinal Involvement in Systemic Disease

Systemic diseases, as well as a number of disorders primarily involving an organ system other than the GI tract, may also result in impaired small-intestinal function and result in malabsorption. For example, patients with diabetes mellitus frequently develop diabetic neuropathy, which may involve the autonomic nervous system in the gut. This then results in delayed gastric emptying in patients with diabetes (diabetic gastroparesis) and in impaired small-intestinal motility, which, in turn, may lead to bacterial overgrowth syndrome and steatorrhea.

Diarrhea and steatorrhea are frequent accompanying features of hyperthyroidism, and are caused by dysmotility. The small-intestinal mucosa remains morphologically normal. Treatment of the underlying disease improves the gastrointestinal symptoms. Hypothyroidism, on the other hand, is characterized by dysmotility-induced constipation. Malabsorption can also occur. Correction of the underlying hypothyroidism also leads to the improvement of malabsorption.

Amyloidosis, scleroderma, and dermatomyositis can be accompanied by malabsorption syndrome. The etiology is a motility disorder resulting in bacterial overgrowth in the small intestine.

Systemic vasculitis with small-intestinal involvement may influence drug absorption owing to either altered motility or mucosal damage. The decreased absorption of diazepam, phenytoin, and acetaminophen was attributed to inflammatory and vascular changes in the duodenum in Behcet's syndrome, even in the absence of clinical evidence of a malabsorption syndrome (12).

Pancreatic insufficiency is also often associated with malabsorption. The most frequent cause of exocrine pancreatic insufficiency is chronic pancreatitis, and in 75% of these patients the disease is due to chronic alcoholism of long duration. In chronic pancreatitis, the pancreas may be enlarged or atrophic, and dilated ducts are filled with thick protein-rich fluid. Protein plugs formed in the smaller ductules may calcify and are thought to initiate a recurrent cycle of obstruction, inflammation, and fibrosis. The ultimate result of the disease is exhaustion of the reserve of exocrine pancreas, cessation of the secretion of the part of the pancreatic juice that is rich in proteolytic and lipolytic enzymes, and

maldigestion. Patients with exocrine pancreatic insufficiency also exhibit decreased pancreatic bicarbonate secretion. As a result, duodenal pH is reduced after a meal, leading to inactivation of orally administered exogenous enzymes and decreased micellar solubilization of bile salts. Treatment of pancreatic insufficiency consists of high-dose enzyme replacement therapy with or without gastric acid suppression; this may reduce clinical symptoms and improve malabsorption, but may cause additional problems for drug absorption and interactions (13).

Drug- and Irradiation-Induced Malabsorption

There are numerous reports on various chemically and pharmacologically different substances that can induce malabsorption syndrome. For example, cholestyramine is a drug of choice in the treatment of cholegenic diarrhea, but because of its high bile acid-binding capacity, it can reduce their content in the gut, with subsequent impairment of the micellar phase of fat digestion. A direct adsorption of ionizable drug onto the resin could also lead to decreased availability for absorption.

Neomycin and kanamycin also cause reduced absorption of fat, proteins, carotene, vitamin B₁₂, and glucose. Neomycin-induced lactase deficiency is typical.

Together with oral antidiabetic drugs, biguanides may cause malabsorption leading to an impaired absorption of carbohydrates, amino acids, bile acids, and vitamin B₁₂ (14). The pseudotetrasaccharide acarbose, a competitive inhibitor of α -glucosidases in the intestinal mucosa, leads to malabsorption of carbohydrates (meteorism, flatulence, diarrhea). *p*-Aminosalicylic acid (PAS) may cause steatorrhea and impaired absorption of vitamin B₁₂, folic acid, and iron.

Irradiation enteritis occurs less frequently than colonic lesions after radiotherapy. Adhesions in the ileocecal area after irradiation therapy of gynecological malignancies may lead to watery diarrhea.

Aging

Although there is little clinical evidence that significant malnutrition occurs in any normal elderly person as a result of the aging process itself (15,16), almost all diseases that may cause malabsorption occur in the elderly. Malnutrition resulting from chronic congestive heart failure (cardiac cachexia) is relatively common. Impaired absorption of fat is related to the clinical severity of heart failure but is apparently not associated with small-bowel bacterial overgrowth (17). Malabsorption in the elderly can be caused by gastric hypochlorhydria, with subsequent small-bowel bacterial overgrowth, or by gastrointestinal dysmotility caused by subclinical hypothyroidism. Moreover, a true defect in calcium absorption in the elderly has been described (18).

Small-Bowel Resection

Short-bowel syndrome is defined as a series of metabolic and nutritional events developing after an extensive intestinal resection. Surgical removal of up to approximately 50% of the small intestine can be well tolerated, because the remaining intestine adapts to an increased demand to absorb nutrients. However, intestinal adaptation takes place only when enteral feeding is used to stimulate the intestinal epithelium to hyperproliferate either directly (owing to

the effect of nutrients themselves) or indirectly by stimulating pancreaticobiliary secretions and by hormonal mechanisms. Resection of 70% to 80% of the small intestine results in severe malabsorption.

Malabsorption in small-intestinal resection is caused by a variety of factors:

1. Marked reduction of the absorptive surface
2. Gastric acid hypersecretion, resulting in pancreatic lipase inactivation and fat maldigestion
3. Reduction of bile acid pool below the amounts necessary for critical micellar concentration
4. Stimulation of colonic secretion by hydroxy fatty acids produced by bacterial hydroxylation of nonabsorbed fat

In the early postoperative phase, small-bowel resection results in a severe watery diarrhea with global malabsorption. During the intermediate phase after surgery, steatorrhea will occur, with subsequent weight loss and malabsorption of fat-soluble vitamins, essential fatty acids, and trace metals. In the late postoperative phase, intestinal adaptation mechanisms are fully operative and, if enough small intestine is left, the symptoms may gradually normalize. However, intestinal adaptation may not be adequate to sustain overall nutrition without supplementary, intermittent, or continuous parenteral support. Because the mucosal absorptive area may be drastically reduced, absorption of orally administered drugs can be seriously diminished in patients with a small-bowel resection.

Clinical sequelae of small-bowel resection depend on the extent of the resection, length of the residual small bowel, health of the remaining intestine, site of resection, presence or absence of colon, and time after resection. The intestinal adaptation will occur several weeks after surgery and will involve changes in small-bowel structure, cytokinetics, and digestive-absorptive function.

In summary, many diseases with primary loci elsewhere in the body as well as local gastrointestinal problems can lead to maldigestion and malabsorption. This can result in changes in release of drug from the dosage form and transport of drug through the intestinal mucosa. Adjustment of drug dosage/formulation or route of administration may be warranted in some patients.

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The Biopharmaceutics Classification System: Recent Applications in Pharmaceutical Discovery, Development, and Regulation

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OVERVIEW

In the early 1990s, research collaborations established between academic scientists and the U.S. Department of Health and Human Services Food and Drug Administration (FDA) focused on the development of new regulatory standards for bioequivalence (BE), to reduce regulatory burden without compromising the quality of drug products. One of the major developments of this effort was the Biopharmaceutics Classification System (BCS) in 1995, which lays the scientific foundation for classifying drugs into four groups on the basis of drug solubility and permeability (Table 1). In BCS, the criteria for high solubility of the drug substance is met when the highest dose can be dissolved in 250 mL of aqueous media or less over the pH range 1 to 7.5 or 1 to 8 at 37°C, as currently stipulated by the FDA and European Medicines Evaluation Agency (EMA), respectively (2,3). High permeability is defined as 90% or more of absorption based on mass balance or compared to an intravenous reference dose (FDA), or as “linear and complete absorption” (EMA) (3). To obtain a biowaiver from FDA, dissolution of the test and reference drug products must be 85% or more of the labeled amount of drug substance within 15 minutes, or alternatively within 30 minutes passing an f_2 test, using U.S. Pharmacopeia apparatus I at 100 rpm or apparatus II at 50 rpm in a volume of 900 mL or less of the following media:

- Acidic medium (e.g., 0.1 N HCl or simulated gastric fluid USP without enzymes)
- A pH 4.5 buffer
- A pH 6.8 buffer or simulated intestinal fluid USP without enzymes

During the past decade, the rationale of BCS has been extensively discussed in the scientific community, effectively implemented by regulatory agencies, and widely practiced by the pharmaceutical industry. These efforts led to regulatory relief including an improved Scale-Up and Post-Approval Changes–immediate release (SUPAC-IR) guidance in 1995 (4), a guidance on dissolution testing of IR solid oral dosage forms in 1997 (5), and a draft of 1999 and subsequently the final version of the guidance for waiver of in vivo bioavailability (BA) and BE based on BCS in 2000 (2).

Today, BCS exhibits a much more remarkable impact on the global pharmaceutical community than its original goal of providing a scientific foundation for regulatory BE decisions. BCS is not only continuously evolving to further improve regulatory BE assessment but also has been revolutionizing the

TABLE 1 BCS Classification and IVIVC Expectations for Immediate-Release Drug Products Based on BCS Class

Class	Solubility	Permeability	IVIVC expectation
I	High	High	IVIVC is expected if dissolution rate is slower than the gastric emptying rate, otherwise limited or no correlation.
II	Low	High	IVIVC is expected if in vitro dissolution rate is similar to in vivo dissolution rate (assuming no or limited precipitation), unless dose is very high.
III	High	Low	Absorption (permeability) is rate determining and limited or no IVIVC with dissolution rate.
IV	Low	Low	Limited or no IVIVC is expected.

Abbreviations: BCS, Biopharmaceutics Classification System; IVIVC, in vivo–in vitro correlation.

Source: Modified from Ref. 1.

process of drug discovery and development in pharmaceutical industry. Further, as a scientific and mechanistic classification tool, BCS has been applied to global drug lists to assist regulatory agencies to efficiently deliver quality essential medicines to the general public (6).

This chapter summarizes the latest applications and development of BCS, including the best practice in classification of new drug entities, regulatory contributions to the waiver of in vivo BE and its extensions, influences on drug discovery and development worldwide, and provisional classification of the drugs present in the worldwide top-selling IR oral drug products.

METHODOLOGIES OF ASSESSING BCS CLASSIFICATION

Biopharmaceutics classification of a drug substance is based on three criteria, namely, the solubility of the drug substance, its permeability, and the dissolution properties of the IR drug product. As the FDA solubility definition of a “high-solubility drug” stipulates use of the highest strength in marketed products to be used in the calculation of the dose:solubility value, assessment of BCS solubility classification is straightforward. Addition of surfactants during the solubility measurement has been proposed to simulate the presence of bile salts in vivo, as in biorelevant media (7). However, the regulatory requirement remains as of this writing conservative in requiring classification based on the lowest solubility in the physiological pH range in water/buffer. As an alternative to standard solubility determination methods, intrinsic dissolution rate (IDR) was investigated as a surrogate method for determining drug solubility class (8). Specifically, 15 model drugs were tested at 100 rpm in 900 mL at pH 1.2, 4.5, and 6.8, and the results suggested that an IDR value of 0.1 mg/min/cm² would give a good prediction of the class boundary except for extreme doses. IDR may therefore be a good alternative method for assessing solubility early in development, when only small amounts of drug are available.

According to the FDA, the permeability classification, which is dependent on fraction absorbed, requires mass balance of human data or intravenous and oral BA data. These data are often unavailable when developing new chemical entities (NCEs). Direct measurement of permeability in situ in humans is the most direct methodology, but is not widely accessible. Therefore, numerous alternative methodologies to assess permeability have been proposed, including in silico calculations and in vitro cell models [such as Caco-2 and Madin-Darby Canine Kidney (MDCK)

cell models]. The in situ permeability measurement in rats is probably the most reliable of these methods for preclinical permeability classification (9).

Recently, Benet et al. have proposed a new approach to estimate the permeability classification on the basis of the extent of drug metabolism (10). The rationale is that the drug must be absorbed before it is extensively metabolized (exceptions would be luminal metabolism or degradation). Specifically, at the highest dose strength, a drug can be classified as high permeability if its phase 1 oxidative and phase 2 conjugative metabolites are equal to or more than 90% of the oral dose. Employing the extent of drug metabolism presents a practical alternative for drugs that are on the market, though it is of less utility for preclinical development due to animal-human metabolism differences (10,11). Benet et al. noted that using 70% metabolism as the cutoff for high or low permeability accurately predicted permeability for the 20 model permeability drugs suggested by FDA, and was 93% accurate for the 29 drugs with measured human permeabilities. Therefore, the criterion of $\geq 90\%$ metabolism is conservative, although analogous to $\geq 90\%$ absorbed in the permeability definition (10).

Relaxation of the dissolution criterion with respect to product "rapid dissolution" has been suggested beyond the current standard of $\geq 85\%$ within 30 minutes (7). Specifically, it has been proposed that the boundary of $\geq 85\%$ within 15 minutes or 30 minutes may be too conservative. Particularly for BCS class I and II drugs (high permeability) that are absorbed throughout the intestine, products with longer in vivo dissolution times may still be able to meet the $C_{\max}$ and AUC requirements for BE. Moreover, the ideal in vitro dissolution methodology should detect differences on the in vivo performance of products and be supportive of regulatory review rather than aiming for maximum discrimination between products (12,13).

WAIVER OF IN VIVO BE AND ITS EXTENSIONS

BE studies are the critical tool that connect the drug product with the clinical benefits claimed in the labeling. With BE, the same clinical results are ensured for the innovator products and for the generic products or those that have undergone various manufacturing changes. The current BE standard is essentially empirical, based on plasma levels and employing a relative BA approach to BE. Specifically, the FDA approves BE if the 90% confidence intervals of $C_{\max}$ and AUC of the test product fall into the 80% to 125% range of the innovator product. According to the FDA CFR 21.320.1 definition, BA means "the rate and extent to which the active drug ingredient or therapeutic moiety is absorbed from a drug product and becomes available at the site of drug action." Therefore, plasma data collected during the BE studies serve as surrogate of BA. However, the true BE should focus on the term "is absorbed," because it is the absorption that leads to subsequent systematic availability.

BA and BCS approaches to BE are fundamentally different from one another. BCS opens a new mechanistic BE paradigm based on two key parameters controlling the in vivo drug absorption process, that is, solubility and permeability. It is expressed in equation (1) in mathematical terms as follows:

$$M(t) = \int_0^t \iint_A P_W C_W dA dt \quad (1)$$

According to equation (1), for two drug products containing the same active pharmaceutical ingredient (API) that can be absorbed from a given surface area, A , it is assumed that if they have the same permeability and concentration-time profile at the intestinal mucosa (gut wall), they will have the same extent and rate of absorption and therefore be bioequivalent. According to this paradigm, permeability, solubility, and dissolution, rather than the plasma levels, are the factors that determine BE, and thus generate any real differences in the in vivo performance between two drug products. It should be emphasized here that while permeability is the fundamental parameter controlling the rate of drug uptake from the small intestine, the dissolution in vivo controls the presentation of the drug to the mucosa in the small intestine. Thus, BE decisions can be based on in vitro dissolution rather than in vivo human BE studies for qualified drug products. This is the basis for the regulatory waiver of in vivo BE through the scientific and mechanistic rationales provided by BCS. BCS recommends in vitro dissolution testing in lieu of in vivo BE studies, and is thus essentially a different approach to establishing BE rather than a waiver of BE studies per se.

The implementation of the BCS approach to BE has greatly facilitated and accelerated new drug development, particularly in phases 2, 3, and 4 of clinical development, as well as in the regulatory approval of changed or generic drug products. Its implication in SUPAC is one good example. According to the BCS approach, formulation and processing changes will not influence in vivo BA of a BCS class I drug formulated in IR dosage forms if the products under comparison both meet FDA criteria (2,4). A study with propranolol and metoprolol, both highly soluble and highly permeable (BCS class I) drugs, confirmed this approach. The two APIs were manufactured as small and large batches on scale of 6 kg versus 60 kg, and 14 kg versus 66 kg, respectively. Their in vitro dissolution profiles were established according to the FDA guidance and then compared with those of innovator products. Even though lower dissolution rates were observed for the large batches, they still reached more than 85% of drug release within 30 minutes. According to both the FDA and EMEA, increases or decreases in batch size can be approved with biowaiver for BCS class I APIs. In fact, in vivo human studies demonstrated that both 90% confidence intervals of $C_{\max}$ and AUC for smaller and larger batches fell within the 80% to 125% range of the innovator products for both drugs (14).

Biowaiver of BCS class I compounds has been practiced in both innovator and generic companies. Applications utilizing the BCS approach to waive in vivo BE studies have been approved in at least 12 cases by the U.S. FDA (15). One example is pregabalin, developed by Pfizer (Kalamazoo, Michigan, U.S.A.). Pregabalin is a highly soluble compound with a minimum solubility of 33 mg/mL over the pH range of 1 to 7.5 at 37°C. In addition, pregabalin demonstrates high permeability with an extent of absorption of more than 90% (16). The in vitro dissolution rates of all capsule formulations met the rapidly dissolving criteria, that is, more than 85% within 30 minutes. FDA approved the biowaiver for pregabalin capsules in phase 3 development (17), which certainly shortened the submission timeline and eliminated the costs associated with in vivo BE studies. In the generic industry, Mylan Pharmaceuticals (Morgantown, West Virginia, U.S.A.) has filed several Abbreviated New Drug Applications (ANDAs) requesting waiver of in vivo BE studies based on BCS (18). In Europe, the German regulatory authority (BfArM, Bonn) has granted approval of a sotalol hydrochloride generic product based on the BCS class I approach (19).

The highest dose strength of sotalol (160 mg) is soluble in 250 mL of aqueous buffers at pH 1, 4.5, 6.8, and 7.5. Further, Caco-2 permeability studies and absolute human BA of $\geq 90\%$ suggest that sotalol falls in the high-permeability class. Additionally, the generic drug products demonstrated $\geq 85\%$ drug release within 15 minutes. The Swedish regulatory agency, Medical Products Agency (MPA), has approved phenoxymethylpenicillin potassium, prednisolone, tranexamic acid, paracetamol, and (RS)-ibuprofen drug products based on the BCS (20). Among these phenoxymethylpenicillin potassium (21,22), prednisolone (23), and paracetamol (24) are BCS class I drugs. Therefore, when these drug products fulfill EMEA requirements, biowaivers are granted. Interestingly, tranexamic acid is a BCS class III class drug. Its maximum dose 1.5 g is freely soluble in 250 mL buffer within pH 1 to 6.8. It has linear pharmacokinetics with a 55% of fraction absorbed (25). Because the generic products demonstrate similar dissolution profiles with the innovator product, with more than 85% drug release in five minutes within pH 1.2 to 6.8, MPA approved the biowaiver for the 500 mg dose strength. Finally, (RS)-ibuprofen is classified as a BCS class II acidic drug. The extension of biowaiver to BCS class II and III drugs will be discussed further in a later section of this chapter.

The biowaiver for BCS class I drugs could be further extended to the fed state. Interestingly, for ANDAs, FDA recommends BE under fed state, even though this is not required for innovator products. Food effects are least likely to impact BE of BCS class I drugs formulated in IR drug products. This is because for a BCS class I drug, its oral absorption is usually pH and site independent and thus insensitive to modest dissolution differences, including food-induced differences (2). Therefore, rapidly dissolving formulations containing BCS class I drugs could qualify for waiver of in vivo BE in fed state. For instance, metoprolol generic products with statistically significant differences in dissolution profiles yet meeting the FDA BCS guideline were selected to test the effects of food on BE. When administered with the standard FDA breakfast, the 90% confidence intervals for generic metoprolol products were 98% to 118% for the $C_{\max}$ and 92% to 115% for the $AUC_{\inf}$ (26). Pregabalin is another BCS class I example where food does not significantly affect the extent of absorption on the basis of AUC data using FDA confidence interval criteria (17). Recently, it has been reported that BCS class I drugs may experience negative food effects, such as those observed for ceftibuten and hydralazine (27). However, the aqueous solubility of ceftibuten dihydrate was reported to be less than 0.1 mg/mL at 20°C (28,29), which places ceftibuten in BCS class II at the 400-mg dose level. More importantly, it should be noted that the food effects were studied solely with the innovator capsule formulation (30). Therefore, the reported decreases of approximately 33% in $C_{\max}$ and 20% in AUC reflect a negative food impact on BA of the drug rather than a BE difference between drug products. Determining the effect of food on BE requires comparing two drug products containing the same API, therefore, for the study of food on BE, it would be necessary to compare the pharmacokinetics head-to-head in both the fasting and fed states. For hydralazine, the negative food effect on BA has been suggested to be associated with a transient increase in hepatic blood flow and intravascular conversion of hydralazine to pyruvic acid hydrazone (31,32). If this is the case, the root cause is the interaction of the drug with human physiology and not a formulation effect. Hence, it is expected that food effects on the test and reference drug products would be similar.

Extension of the BCS-based biowaiver to other BCS classes has attracted extensive scientific interest. An example is when a dosage form containing a higher dose falls into the low-solubility category according to FDA criteria, and consequently can no longer be classified as BCS class I. For lower strengths, however, the same API is “highly soluble” and therefore qualified for the BCS class I. For instance, the FDA Orange Book lists the approved doses for diazepam of 2, 5, and 10 mg. With a Clog P value of 2.98 and a solubility of 0.01 mg/mL (33), the 2-mg dosage form renders diazepam as a highly soluble compound (BCS class I), whereas 5 mg and 10 mg diazepam have lower solubilities (BCS class II). Since the FDA solubility boundary is conservative, biowaivers for diazepam at the lowest strength may be warranted and perhaps even that at higher strengths.

BCS biowaiver extension to the BCS class II acidic drugs has been extensively debated (7,34,35). It has been suggested that the FDA high-solubility definition for BCS class II acids are too restrictive (34). For BCS class II acidic drugs with pK_a values within the pH range of the gastrointestinal (GI) tract, their solubility and the subsequent dissolution is low at stomach pH 1.2 to 2.1 to qualify as highly soluble: they do not meet the FDA high-solubility standard across the entire pH range of 1 to 7.5. However, because of the upward shift in pH upon entry into the small intestine to pH 4.4 to 6.4, their solubility increases dramatically and so does their dissolution rate. Therefore, the BCS class II acids may behave similarly to the BCS class I drugs, demonstrating high solubility, high permeability, and rapid in vivo dissolution. In fact, numerous BCS class II acids such as indomethacin (36,37), ketorolac (38), and ketoprofen (39,40) exhibit almost complete absorption, with more than 90% BA in humans. Thus, BCS class II acids that are sufficiently soluble at intestinal pH can be scientifically justified for waiver of in vivo BE studies. Indeed, the World Health Organization (WHO) has recommended waiver of in vivo BE studies for BCS class II acids if they fulfill the following criteria: (i) API solubility is highly soluble at pH 6.8 although not at pH 1.2 or 4.5, (ii) the multisource (generic) and comparator products are rapidly dissolving with 85% or more dissolution within 30 minutes at pH 6.8 under 75-rpm paddle or 100-rpm basket, and (iii) dissolution profiles of the multisource and comparator product are similar according to f_2 evaluation at all three pH values (pH 1.2, 4.5, and 6.8) (41,42).

BCS class III biowaivers have also been extensively considered and recommended by several scientific workshop reports. The scientific rationale for biowaiver of BCS class III drugs is the following: BCS class III drugs have high solubility, so if their IR dosage forms dissolve rapidly, the overall absorption will be controlled by gastric emptying and the drug permeability and is thus independent of formulation. As for BCS class I compounds, rapidly dissolving formulations can be considered to behave like oral solutions, which do not require BE testing. Remarkably, WHO has followed this rationale and has recommended the biowaiver procedure for BCS class III drugs, provided both the multisource (generic) and comparator product are very rapidly dissolving with 85% or more dissolution within 15 minutes at pH 1.2, 4.5, and 6.8 using 75-rpm paddle or 100-rpm basket (41,42). In reality, this dissolution criterion has been shown to be conservative in waiving of in vivo BE of IR products containing a BCS class III drug, such as cimetidine (43). Using Tagamet 400 mg tablet as the reference, three 400-mg cimetidine tablets were formulated with 7.5%, 15%, and 26% of methacrylate copolymer, yielding significantly different in vitro release

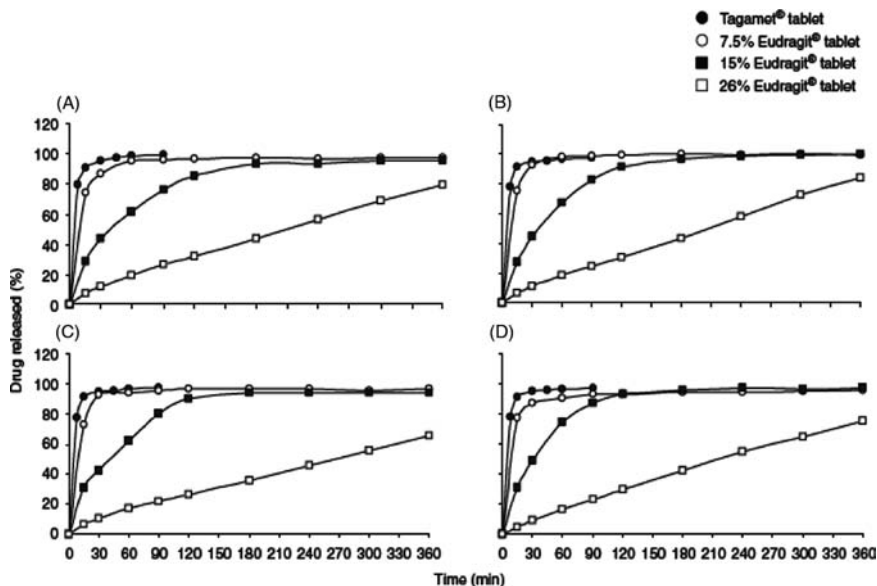


FIGURE 1 Comparison of mean cimetidine released-time profiles obtained from dissolution testing of cimetidine tablets containing methacrylate copolymer and Tagamet[®] tablets in different media. Each represented value is the mean of six observations: (A) 0.01 N HCl, pH 2; (B) phosphate buffer, pH 4.5; (C) SIFsp, pH 6.8; and (D) fasted-state simulated intestinal fluid (FaSSIF), pH 6.5. Source: From Ref. 43.

profiles (Fig. 1). The cimetidine tablet containing 7.5% methacrylate copolymer exhibited more than 85% release within 15 minutes, and in vivo human results showed that it is bioequivalent to the Tagamet reference. More interestingly, although the cimetidine tablet with 15% copolymer reached more than 85% release only after 120 minutes, in vivo human results proved that it also is bioequivalent to the Tagamet reference tablet (Fig. 2). Therefore, for these two cimetidine tablets, permeability rather than the dissolution rate controls the overall absorption. In comparison, the cimetidine tablet with 26% copolymer delayed the drug release rate so significantly that the dissolution rate became the rate-limiting step for absorption.

While scientifically justified and clinically supported, the implementation of biowaivers for BCS class III has been slow. This has probably been due to the concerns about potential excipient effects. While excipient effects on permeability have been shown in *in vitro* Caco-2 cells, extrapolation of these effects from *in vitro* to *in vivo* has a number of uncertainties, and few *in vivo* effects have been documented. The nonconventional excipient sodium acid pyrophosphate (SAPP) was used to investigate its impact on the BA of ranitidine (44). Specifically, 150-mg ranitidine oral solution was single dosed to health volunteers, with or without coadministering 1132-mg SAPP. The results, based on AUC data, indicated that ranitidine absorption was 54% in the presence of SAPP. A subsequent scintigraphic imaging study suggests that without altering gastric emptying time SAPP decreased small intestine transit time by 56% (44). Especially for BCS class III drugs with potential regional dependent permeability, dissolution must occur

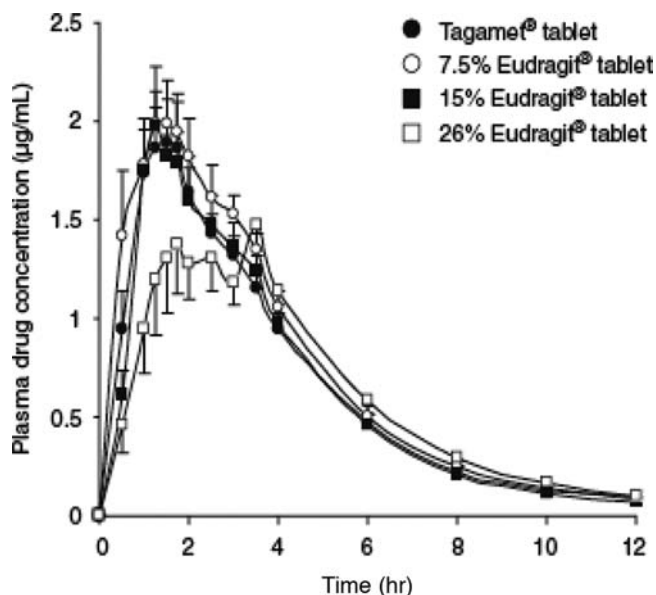


FIGURE 2 Comparison of mean plasma cimetidine concentration–time profiles obtained after administration of a single oral dose of cimetidine tablets containing methacrylate copolymer or Tagamet tablets. Each point represents the mean plasma cimetidine concentration (standard error) from 12 subjects. *Source:* From Ref. 43.

rapidly to ensure maximum absorption, which begins in the duodenum and continues to the ileum. Therefore, the evaluation of excipient effects on permeability must come from direct permeability measurements *in vivo* rather than from the indirect evidence from tissue cultures.

Although *in vivo* BE studies have served as the widely accepted “gold standard,” linking the physical product to the label claim and then to its clinical performance, it can be argued that *in vitro* dissolution testing as the surrogate approach can be superior to traditional human BE studies. In addition to cost reduction, operational convenience, and ethical benefits, *in vitro* testing directly examines the drug release from drug products, which is the focus of BE, whereas the *in vivo* BE studies measure *in vivo* drug release indirectly through systematic availability (45). In addition, for drugs with high *in vivo* variability, an unreasonably high number of human subjects is required to demonstrate the true 90% of confidence intervals. For example, in the case of nadolol, the $C_{\max}$ is very sensitive to the individual absorption rate, resulting in relatively large variability of $C_{\max}$. Thus, for nadolol IR drug products, $C_{\max}$ measurement through conventional human BE studies may be an inefficient tool for assessing BE (46).

BCS IN DRUG DISCOVERY AND DEVELOPMENT

In today’s pharmaceutical industry, the concept of BCS is being widely employed and plays various and important roles at every stage of drug discovery and development. At the stage of lead selection and optimization, along

with the criteria of compound potency and target specificity, compound solubility and permeability are used to assess the potential “developability” and provide a rank order of the compounds. Later, in phase 0 and the preclinical setting, in addition to consideration of compound preformulation characteristics such as its crystallinity, polymorphism, and stability, BCS plays a significant role in the selection of solid forms. In addition, BCS provides guidance for developing early formulations to be dosed in animals. Achieving high solubility in vivo is central for poorly soluble compounds, where exposure requirements for safety assessment are especially challenging. Formulation practices such as cosolvent solubilization, pH modification, polymeric complexation, lipid assemblies, as well as chemical approaches such as prodrugs are essentially used to reduce the dose number [$D_0/(C_s \times 250 \text{ mL})$, D_0 is the dose and C_s is the solubility] in vivo, thus reducing solubility limitations to absorption. The pro-drug approach has been reported to enhance the solubility of poorly soluble parent drugs, as extensively reviewed by Stella et al. (47). For example, carbamazepine is a BCS class II drug with a poor aqueous solubility of 120 $\mu\text{g/mL}$, whereas its sulfonamide derivatives show significant enhancement in apparent solubility to cover 100 mg/mL (with final pH of 2.6) (48). Investment in approaches to reduce dose number at this early stage may seemingly increase the cost and slow down the timeline of discovery, but certainly helps to decrease the attrition rate in later phase of development (49).

For the design of first-in-man formulation (50), BCS classification helps to identify and thus overcome any solubilization and/or permeability challenges, and thus maximize oral absorption and subsequent systemic exposure. In phase 2a development, BCS continues to provide the framework for directing the formulation development strategies because BCS identifies the rate-limiting step to oral drug absorption (51,52). For example, for a BCS class I compound developed into an IR dosage form, there is wide flexibility in the selection of formulations and processes that can ensure a desirable absorption profile. By comparison, for a BCS class II compound intended for immediate release, the choices of formulation and process would be focused on enhancement of in vivo solubility and dissolution. Therefore, formulation technologies such as solid dispersion and lipid formulations, and process technologies such as particle size reduction and hot melt extrusion are utilized to increase the in vivo dissolution rate, thus improving the rate and extent of drug absorption.

For a BCS class III compound, the permeability is the rate-limiting step for overall absorption. Two approaches are commonly used in the industry to enhance the absorption of a BCS class III compound. One approach is the pro-drug strategy, which has attracted numerous research interests and is employed to improve the passive or transporter-mediated intestinal permeability. For example, ester prodrugs of carboxylic acids such as simvastatin, lovastatin, and fosinopril can improve the passive intestinal permeability (53,54). In addition, by utilizing nutritional transporters such as PEPT1, amino acid ester prodrugs have also been successful. For example, valacyclovir is a valine ester prodrug that exhibits a fivefold higher oral BA than acyclovir. This is the combined result from an increased transport by the intestinal dipeptide transporter hPEPT1 and a subsequently activated cleavage by the novel nucleoside prodrug-activating enzyme biphenyl hydrolase-like protein (55–57). The prodrug approach requires active collaborations across discovery team and development scientists. But due to the significant increase in cost and timeline, the prodrug approach is reserved

for substances that are difficult to address with other strategies. The other approach to enhance drug permeability is through addition of excipients in formulation. Utilization of pharmacological effective agents such as occludin and claudin to improve drug transport through tight junction is possible; however, this approach has significant regulatory complications (58). The feasibility of using common excipients such as surfactants has been demonstrated in *in vitro* models at cell levels; however, its utility *in vivo* or at the regulatory approval level is highly questionable. (22). This is mainly due to the concerns of the specific target and the diluting effect of the GI fluids and the residence time issues generated by the GI motility (59).

In phase 3 and 4 stages of clinical development, *in vivo* BE studies are conducted for New Drug Applications (NDAs) and supplementary NDAs for innovator companies, for ANDAs for generic companies, and for SUPAC in the entire pharmaceutical industry. In all these scenarios, BE is essential to successfully bridge clinical formulations to commercial formulations (2,52). Along with formulation design, BCS directs the BE strategy: (i) for BCS class I drugs biowaivers should be considered, (ii) for BCS class II and IV drugs a high risk of bioinequivalence may be present, and (iii) for compounds with low and pH-dependent solubility additional considerations may be required.

Food-effect studies are required for BE studies in ANDAs when the labeling specifically indicates that the product can be administered with meals (60). In this regard, BCS has been used as the basis to understand the mechanisms and magnitude of food effects on drug absorption, BA, and BE. Fleisher et al. presented a comprehensive review of food effects on the absorption processes of various drugs (61). Food effects arise in the following three ways: through the food content itself, such as fat and viscosity (62); through interactions between food and drug molecules such as binding, adsorption, stability, and complexation; and through induction of food effects on GI physiology such as GI transit time, bile salt secretion, pH changes, splanchnic blood flow, and passive and active permeability adjustments. All these factors can be overlaid on the BCS framework, that is, the rate-limiting steps in oral drug absorption, with further analysis leading to prediction and understanding of various and complex interplays between GI physiology, foods, and drug products. For example, food effects on BCS class II drugs should be focused on factors affecting the solubility and dissolution rates of the drug in GI tract. Using the classical Noyes–Whitney equation

$$\frac{dM}{dt} = \frac{DS}{h}(C_s - C_t)$$

where dM/dt is the dissolution rate in mass/time, D is the diffusivity of drug molecule, S is the surface area available for dissolution, h is the diffusional layer thickness, C_s is the drug solubility, and C_t is the drug concentration at time t , the solubility and dissolution rate can be increased by solubilization of food fat intake, by GI fluid volume and biliary secretion, and by prolonged gastric emptying time (61). Numerous relevant examples with BCS class II compounds have been published, including albendazole, danazol, efavirenz, griseofulvin, and haloperidol (27). It should be noted here that food effects must be evaluated in the context of clinical doses. For example, when BCS class II drugs such as temafloxacin (63) were given at doses less than their maximum absorbable dose, no food effects were observed. One plausible explanation is that when the low doses are significantly lower than the maximum absorbable doses, absorption is

limited primarily by the dissolution rate rather than by drug solubility (64). Food intake is expected to increase drug solubility dramatically through the solubilization effect. However, the enhancement on dissolution rate is much less significant, mainly because of the much higher aggregate weight of the micelle form and the resulting smaller diffusivity of micellar species (65,66). Diverse and sometimes contradictory observations on food effects on poorly soluble weak bases (BCS class II) have been reported. In general, it can be expected that the meal would elevate the pH in the stomach (67–69), resulting in a reduced dissolution rate of weak bases and subsequent negative food effects. Indinavir is such a drug. Coadministration of a high-fat breakfast with indinavir led to decreased absorption, with AUCs of 6.86 $\mu\text{M}\cdot\text{hr}$ in the fasted state versus 1.54 $\mu\text{M}\cdot\text{hr}$ in the fed state (70). Carver et al. observed negative food effects, specifically that the AUC decreased by 68%, 45%, and 34%, and the mean C_{max} decreased by 74%, 59%, and 46%, for protein, carbohydrate, and fat meal treatments versus fasted control, respectively ($p < 0.05$) (62). However, positive food effects have also been observed with BCS class II weak bases, such as itraconazole. Van Peer et al. observed that, in comparison with an oral solution, the relative BA of itraconazole capsules averaged 39.8% in the fasting state but 102% in the postprandial state (71). Zimmermann et al. reported that the BA of itraconazole was 86% (90% confidence intervals of 65–102%) after a meal, in comparison to 54% (41–77%) in the fasted state (72). This is probably because a longer GI residence time in the fed state leads to an increase of total dissolved amount in small intestine. Additionally, at dose of 100 mg, itraconazole exhibits a dose number around 10^6 , suggesting a solubility-limited absorption process. The intake of high-caloric foods generally induces the secretion of bile salts, which would significantly improve the drug solubility in GI fluids and subsequently promote drug absorption. Taken together, they overcome the effects of poor dissolution at elevated pH in the stomach.

For poorly permeable drugs such as BCS class III compounds, effects of food intake on permeability can be either positive or negative. For example, coadministration of meal with LY303366 showed a negative food effect on AUC (0–48 hours) in dogs. It was proposed by the authors that the regionally dependent permeability of LY303366, which has higher permeability in the upper small intestine (73), was responsible for the effect. Various other possible mechanisms for the observed negative food effect, such as the volume of fluid administered, the prolonged gastric residence time, the meal viscosity, drug-food binding, and food-induced biliary secretion were also discussed but have not been resolved as yet. On the other hand, food can also promote drug absorption through regulation of transporters. Gabapentin is such an example. The C_{max} and AUC (0–6 hours) of gabapentin in rats are significantly enhanced in the presence of glycyl-glutamate through a trans-simulation mechanism (74,75), that is, stimulation of PEPT1 transporter by Gly-Glu dipeptide.

Recently, with the use of logistic regression, the key physicochemical parameters contributing to food effects have been identified, these being the dose, solubility, and permeability (27). Other parameters such as polar surface area, total surface area, percent polar surface area, and the number of hydrogen bond donors and acceptors are surprisingly found to have no significant contribution to food effects. This report further echoes the importance and significance of using the fundamental elements presented by the BCS in considering food effects (27).

In recent FDA initiatives of Quality by Design (QbD) and Quality-based Review (QbR), it is suggested that the key biopharmaceutic properties of the drug substance are recommended to be integrated into the product development. For example, in QbD, a quality system is needed to link the formulation and manufacturing attributes with the desirable clinical performance. In fact, BCS readily presents the in vitro dissolution testing as one of the key and simple tools to ensure satisfactory clinical quality. Recently, in vitro dissolution methodology has been established to build a QbD package for an AstraZeneca class II drug product (76). BCS identified in vivo dissolution as the rate-limiting step of absorption, and the API formulation and process variables were designed to modify the in vitro dissolution behavior of medicinal products. Clinical studies confirmed that the in vitro dissolution methodology served as an effective tie between the desired clinical outcomes and flexible manufacturing sectors (76).

It is noted that the success of discovering a drug candidate and launching the drug product requires multilatitude considerations of potency and safety, manufacturing feasibility and cost, regulatory opportunities and hurdles, and market competition and benefits. All of these factors must be weighted appropriately in addition to the input of BCS.

PROVISIONAL BCS CLASSIFICATION OF TOP DRUGS ON THE GLOBAL MARKET

On the basis of ensuring a similar absorption between drug products, BCS revealed the mechanistic understanding for BE studies. The BCS approach suggests that for a considerable number of drugs formulated in IR dosage forms, the less expensive and faster in vitro dissolution testing rather than the expensive and lengthy human studies is sufficient to assure in vivo BE. Using publicly available databases, such as the WHO Essential Drug List, drugs have been provisionally classified using BCS classification system (33). In this report, aqueous solubility was based on readily available data in literature (Merck Index and USP) and the permeability classification was based on the correlation of human intestinal membrane permeability of a set of 29 references drugs with the estimated $\log P$ or $\text{Clog } P$. A high-solubility drug is defined as one for which the dose number D_0 using the maximum dose strength and lowest solubility reported ($D_0 = \text{maximum dose}/250 \text{ mL}/\text{solubility}$) is 1 or lower. Employing $\log P$ and $\text{Clog } P$, 23.6% and 28.5% of 123 WHO oral drugs were assigned in BCS class I, which are candidates that are certainly qualified for biowaivers, according to the FDA, CPMP, and WHO. In addition, approximately 30% of WHO drugs belong to BCS class III, which are recommended by WHO for biowaiver (41,42) and are potential candidates for regulatory approval of generic drug products using biowaiver approach in individual countries in the future. More recently, using a similar approach, the top 200 oral drug products in the United States, United Kingdom, Spain, and Japan were provisionally classified (77). On these four lists, 55% to 59% of the drugs were determined as high solubility, while 62% to 69% and 56% to 60% of drugs were estimated as high permeability, based on $\log P$ and $\text{Clog } P$, respectively. About 30% drugs were classified as BCS class I on the U.S, U.K. and ES lists and 34.5% on the Japanese list due to the use of 150 mL in calculation. Combined with BCS class III drugs, more than 55% of the drugs formulated in orally administered IR dosage forms were classified as high solubility, which are the first-line potential candidates for biowaiver.

It is well recognized that human intestinal permeability data is relatively limited. In addition, at the very early stage of drug discovery, very little drug substance is available for definitive assignment of BCS classification through a full evaluation. Therefore, tentative BCS classification can be assigned based on *in silico* approaches. In general, the computational approach should be evaluated very cautiously, especially with respect to the underlying assumptions and methods in the calculations. However, due to the convenience and feasibility during early stage development, the *in silico* approach is attracting increasing interest. Recently, in a set of 185 drugs worldwide, *in silico* solubility estimates using the melting points of nonionized drugs showed that a total of 98 drugs could be classified as high solubility and 87 as low solubility. This is remarkably close to the classification of 92 of the 98 drugs as high solubility and 93 drugs as low solubility (solubility data for 92 substances was available from the Merck Index, USP, and other references). In addition, the *in silico* permeability approach using Clog *P* (fragment methods in BioLoom and ChemDrw), log *P* (contribution methods in Molecular Operating Environment), and Klog *P* (molecular formula and contribution from simple element type) demonstrates that it is correct for 21 to 22 of the 29 human permeability reference drugs and 12% to 13% of the 14 FDA permeability reference drugs (78). This work suggests that if the *in silico* method could be validated, it would be a convenient, efficient, and cost-saving approach in the preclinical setting. Further research should be conducted to further improve *in silico* prediction of BCS classification.

Provisional BCS classification can significantly lower the cost and shorten the timeline in bringing generic drug products to the market, in not only developed but also developing countries. This is becoming very significant for the developing countries, where resources and structures for conducting *in vivo* BE studies are scarce. Thus, by implementing *in vitro* dissolution testing for qualified drug products, BCS-based biowaivers can greatly benefit public health worldwide.

CONCLUSIONS

BCS reveals a new paradigm to approach BE significantly reducing regulatory burden based on a mechanistic rationale. Moreover, BCS as a scientific tool has revolutionized the process of drug discovery and development across the pharmaceutical world. Most significantly, BCS contributes to the general health of the public by greatly enhancing the efficiency in drug development and regulatory approval processes.

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INTRALUMENAL SOLUBILITY AND DRUG ABSORPTION

For uptake via the intestinal epithelium to be possible, the drug must be in solution. In most cases, drug absorption takes place in the small intestine and, therefore, the drug concentration achieved in this region is of primary importance. However, the release and dissolution of drug may also be important in other regions of the gastrointestinal (GI) lumen. For example, dissolution in the stomach will affect drug concentrations in the small intestine and, if rapid, will facilitate a fast onset of the therapeutic action. For products that act locally in the colon, the dissolution in that region will dictate the rate and extent of the clinical outcome. Drug dissolution in vivo is difficult to assess, at least on a routine basis (1). Biorelevant dissolution is a useful alternative, but issues relating mainly to hydrodynamics (type and intensity of agitation and volumes) still remain (see chap. 12, this volume).

According to the Noyes–Whitney theory for dissolution (2,3) and its subsequent modifications by Nernst and Brunner (4) and Levich (5), one of the factors affecting the dissolution rate of a solid is the equilibrium solubility, that is, the concentration of the dissolving species in a saturated solution when excess undissolved solid is present. Dissolution rate is proportional to the amount remaining to be dissolved and to solubility (6,7). Biorelevant solubility is the maximum attainable concentration in a specific region of the GI lumen and is useful for estimating the maximum rate of absorption for passively absorbed compounds. Provided that the thermodynamically most stable crystal is used and other parameters affecting dissolution remain constant, intraluminal equilibrium solubilities may also be useful in the comparative assessment of intraluminal dissolution rates and, therefore, can assist in eliminating unsuitable compounds from the drug development process. The high level of interest in the intraluminal equilibrium solubility stems also from the simple setup required to experimentally determine solubilities [even high-throughput procedures can be implemented (8)], its sensitivity to medium composition [even in situations where no direct interaction of the dissolved species with the components of the medium is expected (Fig. 1)], and from its usefulness in the evaluation of the biorelevance of media simulating the luminal environment.

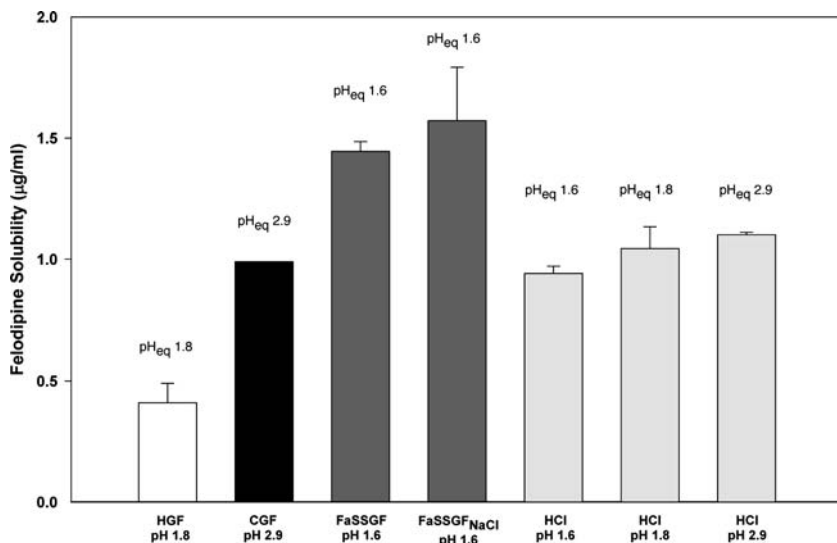


FIGURE 1 Solubility of felodipine (nonionizable) in HGF, CGF, in FaSSGF containing various amounts of NaCl, and in various HCL solutions. *Abbreviations:* HGF, human gastric fluid; CGF, canine gastric fluid; FaSSGF, fasted state-simulating gastric fluid; PHeq, pH at equilibrium. *Source:* From Ref. 9.

Although equilibrium solubility is, in most of cases, the parameter that drives the intraluminal dissolution rate, drug concentration at the gut wall may reach even higher values, that is, supersaturation of luminal contents may occur with some drug/dosage form combinations. To date, in vivo assessment of intraluminal supersaturation has not been investigated, but plasma data suggest that it can be induced by formulation approaches and/or the GI pH gradient. Drug delivery systems designed to generate supersaturation include solubilized formulations as well as physically and chemically modified high-energy solid forms (10). For weakly basic drugs, however, even intake of the crystalline powder may result in supersaturation in the small intestine. Prediction of the ability of a specific drug to form supersaturated solutions would be of great value for the development of low-solubility compounds; the use of supersaturation data instead of equilibrium solubility data may have an impact on whether the compound is considered to be suitable for development as an oral product. In simple aqueous solution where the drug is not ionized, molecules that form supersaturated solutions tend to have higher melting points and be less soluble than predicted from their partition coefficients in an octanol-water system (*P* values) (11). Figure 2 shows a group of BCS class II compounds whose kinetic solubilities (i.e., their concentrations in an aqueous solution at the time when precipitation first occurs) would place them into a region close to BCS class I. The in vivo relevance of this approach has very recently been demonstrated by studies showing that supersaturation of itraconazole occurs in human intestinal aspirates (12).

The aims of this chapter are, first, to describe situations where drug solubility in a specific region of the GI lumen would be useful to know and, second, to discuss the usefulness of biorelevant media in predicting drug solubility and

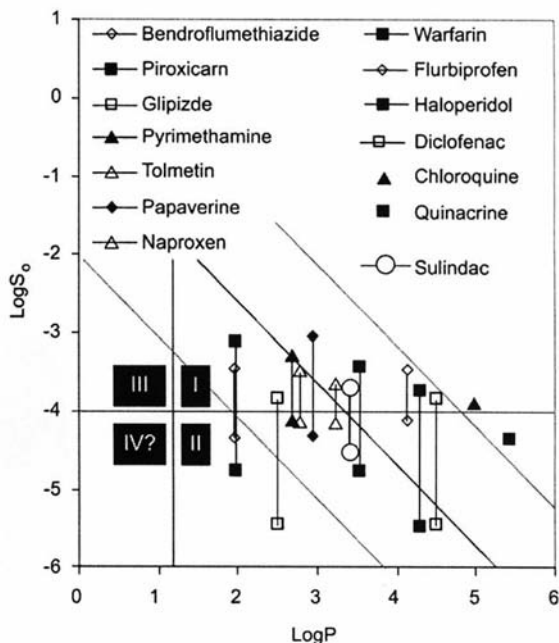


FIGURE 2 Log solubility (Log S_0) data versus log octanol-water distribution (Log P) data of various molecules believed to belong to BCS class II. The vertical lines connect the kinetic and intrinsic solubility values. Although Log intrinsic solubilities are between -4 and -6 , their Log kinetic solubilities are above -4 , in a region where most neighboring molecules are in BCS class I. This graph also shows chloroquine and quinacrine, two compounds which were investigated too late to be included fully in the relevant study (11), which fall in the high solubility region to the right of the outer diagonal line. *Source:* From Ref. 11.

supersaturation/precipitation in the GI lumen. For the colonic region, only a summary of the composition of fluids in the ascending colon will be provided on the basis of recent data.

DRUG SOLUBILITY IN THE FASTED STOMACH

Since in the fasted state 200 mL of water (corresponding to a usual glass of water) is already emptied from the stomach within about 30 minutes, intragastric solubility is of interest primarily

- for compounds that are administered in liquid form from which precipitation in stomach may occur (e.g., lipid dosage forms) and
- for formulations or compounds that are highly soluble and rapidly dissolving in the stomach but that have limited solubility in the small intestine.

The latter applies to supersaturating drug delivery systems and to lipophilic weak bases and their salts (10,13). One issue while measuring the intragastric solubility of a weak base is that the base itself can affect pH, leading to a change in the pH at equilibrium compared to the initial value. This change is a function

of the acidity of the aspirates, the pK_a of the compound to be dissolved, and the concentration of the dissolved ionized species at equilibrium (9). It should be noted that a change in pH might also occur *in vivo* during intragastric dissolution of a weak base, especially if high drug concentrations are reached.

Intragastric solubility may also be of interest for poorly soluble, non-ionizable, or weakly acidic compounds. Such compounds typically dissolve in the small intestine, rather than in the stomach, because of the lack of significant amounts of solubilizing agents in the fasted stomach. Despite the fast gastric emptying rate in the fasted state, agglomeration of solid particles or precipitation of salts of weak acids in the fasted stomach can, in certain cases, greatly influence dosage form performance *in vivo* (14).

Various media have been evaluated for estimating intragastric solubility in the fasted state. On the basis of data with ketoconazole, dipyridamole, and miconazole, canine gastric aspirates lead to underestimation of solubility in human gastric aspirates because of their higher pH (9). In contrast, simulated gastric fluids suggested by pharmacopeias worldwide either lead to an overestimation of intragastric solubility of weak bases [due to their lower pH and/or presence of pepsin (9)] or they lead to an underestimation [as pH of these fluids is lower than the pH in the fasted stomach after 200 mL of water and can be lower than the pH of maximum solubility of a weak base (9)]. Solubility data in fasted state—simulating gastric fluid (FaSSGF) that contains physiologically relevant surfactants and has a pH of 1.6 (15) or solubility data in HCl pH 1.6 provide a comparatively better basis for the assessment of intragastric solubility during a bioavailability study in the fasted state (9). However, since particle agglomeration or drug precipitation rates are also dependent on the surface tension of the fluid, the lower surface tension of FaSSGF may make it more appropriate than HCl pH 1.6 for studying those processes *in vitro*.

Despite the comparative superiority of FaSSGF or HCl pH 1.6, accurate estimation of intragastric solubility remains problematic for two reasons. First, unlike the small intestinal contents in the fasted state, in which mixed bile salt micelles constitute the main solubilizing species, the gastric fluid contains numerous substances, each in minute concentrations, which can contribute to solubilization; therefore, small variations in concentrations of individual components may have a substantial effect on drug solubility (9) (Fig. 1). On the basis of data collected in individual aspirates from five volunteers, both the intra- and intersubject coefficients of variation of solubility of danazol (nonionizable, low-solubility compound) in the gastric contents is about 30% (16). The second reason for inaccurate estimation of intragastric solubility relates to the methodology applied for aspiration. For example, the mean solubility of danazol in aspirated gastric contents of five individuals was measured to be 1.6 $\mu\text{g/mL}$. This value is 3.3 times higher than the solubility of danazol in HCl pH 1.2 containing 34.2 mM NaCl (16). In contrast, the solubility of felodipine (nonionizable, low-solubility compound) in pooled aspirates collected from 12 subjects was 0.4 $\mu\text{g/mL}$ and this value is less than half the solubility in HCl solutions (Fig. 1). The fact that solubility in HCl leads to underestimation of intragastric solubility of danazol but to overestimation of intragastric solubility of felodipine may be attributed to the different protocols applied for aspiration. In the danazol study, no information on the volume of water administered prior to aspirations was provided (16). In the felodipine study, 250 mL of water was administered 20 to 40 minutes prior to aspiration (9), and it can be speculated

that any bile secretions that had been refluxed from duodenum were already washed out of the stomach at the time of aspiration. This hypothesis is supported by the comparatively high bile salt and phospholipid levels in the aspirates in which danazol solubility was measured.

Recently, a medium simulating the contents of the fasted stomach without the use of physiologically relevant components has been proposed (17). This medium may be useful in situations where equilibration times are very long and, therefore, the possibility of denaturation of physiological components may be high. However, the usefulness of this medium in predicting intragastric solubility remains to be evaluated.

DRUG SOLUBILITY IN THE FED STOMACH

In the fed state, gastric residence times of immediate release dosage forms are increased substantially (18), so dissolution of solid particles in the stomach will be more extensive than in the fasted state. As a result, for compounds with dissolution limited absorption rates, the initial rise in plasma levels in the fed state will likely depend on the intragastric dissolution profile (19). Intragastric solubility in the fed state should, therefore, be of interest for BCS class II and class IV compounds. However, since the intragastric environment in the fed state contains various solubilizing agents that are still to be digested, one should be cautious when extrapolating solubility differences to dissolution performance. Because of the substantially lower diffusivity of the solubilizing agents (20–23) and/or the small interfacial area of the high capacity components [e.g., lipid droplets vs. micelles (24)], dissolution rates may not reflect increases in equilibrium solubility. It has recently been shown that these mechanisms are important for the release rate of felodipine from an extended release formulation in media simulating the gastric contents in the fed state (25).

Although for low-solubility compounds solubility in the contents of the fed stomach is expected to increase (due to the increased presence of various components that promote solubility), the extent of such an increase will likely vary with the amount and composition of administered meal and with the time after meal administration. Experimentally, difficulties in aspirating samples after administration of a solid meal make measurement of intragastric solubility challenging. A practical way to proceed is to aspirate samples after administration of a liquid meal that contains nutrients similar (both in terms of type and amount) with those existing in solid meals administered in bioavailability/bioequivalence studies (26). Five hundred milliliters of Ensure plus[®] has been used as a liquid meal that reflects the composition of a standard breakfast, while facilitating the aspiration procedure (27).

It has been proposed that by diluting homogenized long-life milk with buffers and/or by adding appropriate amounts of NaCl to milk, one can prepare “snapshot” media that reflect the pH, buffer capacity, and osmolality of gastric contents early (during the first 75 minutes), in the middle (from 75 to 160 minutes), and at later times (longer than 160 minutes) after ingestion of 500 mL Ensure plus[®] (28). An alternative approach has been proposed, in which the emphasis is placed on the simulation of intragastric secretions in the fed state and, therefore, on the simulation of intragastric lipid and/or protein composition (25). As with the first approach, intragastric conditions in the second approach have been simulated by using milk as the basis for the medium, since

milk's composition is close to the intragastric composition early after administration of meals commonly used in drug absorption studies (27,29,30). Digestion of homogenized, long-life milk is allowed to proceed using biorelevant amounts of hydrochloric acid, pepsin, and lipase. Using this approach, the effects of gastric residence on the performance of certain dosage forms (31) as well as the release profile of felodipine from an extended release formulation in the fed stomach (25) have been predicted. The usefulness of the "nondigested" and the "digested" media versus simple aqueous media having pHs similar to those of the aspirates in predicting intragastric solubility in the fed state has recently been evaluated using dipyrindamole and ketoconazole as model compounds (32). Simple aqueous buffered media vastly underestimated intragastric solubility of the two model compounds in the fed state (32). When using undigested milk-based media, solubilities of model compounds in aspirates were also underestimated by a factor of 2.5 to 27. Solubility in milk digested with pepsin was useful for estimating intragastric solubility of ketoconazole (within 20%) but overestimated intragastric values of dipyrindamole by a factor of 2 to 19. For both drugs, solubility in milk digested with pepsin and lipase predicted the solubility in aspirates collected 60 minutes after meal administration, whereas, at other times, it overestimated intragastric solubility (in this case by a factor of <5). On the basis of the data with dipyrindamole and ketoconazole, both the use of biorelevant media and simulation of intragastric digestion are necessary for prediction of drug solubility in the fed stomach (32). Simulation of vesicle/micellar structures seems to be key for the prediction of intragastric solubility in the fed stomach and, therefore, for the prediction of intragastric dissolution rates. The latter is difficult to confirm experimentally, because of limited availability of human gastric fluids. However, it has been shown very recently that simulation of colloidal structures in the fed stomach is crucial for the prediction of release kinetics of felodipine from an extended release matrix formulation in the fed stomach (25).

Measuring intragastric solubility in the fed state can be a laborious process, especially when digested media are used. One issue is the separation of solid drug particles from the medium. Ultracentrifugation, although seemingly the most promising, usually requires a long processing time during which the concentrations may change. Filtration and centrifugation both can be considered as alternatives (25,32). Another issue is the drug quantification procedure. Construction of standard curves must be done in the same media and using the same treatments applied to the test samples (25). In addition, since composition of aspirates varies with time, standard curve characteristics may also vary with medium composition, and, therefore, standard curves may have to be constructed in more than one medium.

DRUG SOLUBILITY IN THE FASTED SMALL INTESTINE

The small intestine is the primary site of absorption for the majority of drugs. Intraintestinal concentrations provide the driving force for flux through the mucosa and, therefore, the absorption rate. Assuming no stability issues, intraintestinal concentrations depend on the water flux across the intestinal wall, on the concentration of drug in the fluids arriving from the stomach, and on the dissolution of particles arriving from the stomach. The hyposmolarity in the fasted small intestine (e.g., 26) suggests fast water absorption. The drug

concentration in the fluids entering the small intestine from the stomach is of particular interest for poorly soluble weak bases, because they are likely to be dissolved during gastric residence but have the potential to precipitate in the small intestine because of the sharp pH increase. Although intrainstestinal precipitation could potentially complicate absorption of lipophilic weak bases, no conclusive *in vivo* data have been generated to date (33). Nevertheless, some methods have been developed for assessing potential precipitation *in vitro* with the help of biorelevant media. Kostewicz et al. (34) were the first to develop a precipitation model consisting of a two-compartment system simulating the transfer out of the stomach into the intestine. A solution of the drug in simulated gastric fluid is continuously pumped into a simulated intestinal fluid, and drug supersaturation/precipitation in the acceptor medium is examined via concentration-time measurements (34,35). For drugs with a fast absorption rate, this system may overestimate the possibility for precipitation. Subsequently, a four-compartment system was proposed that, as well as the gastric and intestinal compartments, includes an "absorption" compartment and a "reservoir" to replace the volume from the intestinal compartment that is "absorbed" (36). One issue with the second system is validation of the filtering procedure used when separating "intestinal" from absorption compartment. Additionally, for both systems, the volumes used for the intestinal compartments probably still need to be optimized.

For lipophilic compounds, solubility in the lumen of the fasted small intestine is usually far greater than the solubility measured in simple aqueous media with similar pH values. This is primarily due to the presence of mixed bile salt micelles (37,38). However, since distribution of drugs in octanol does not always correlate with the distribution of drugs in mixed bile salt micelles (38,39), solubilities have to be measured in aspirates or surrogate media.

Drug solubility in the fasted small intestinal contents varies with time and with the individual (Fig. 3) (16,39). Both sources of variability in solubilizing capacity correspond to the variability of intraluminal physicochemical characteristics (40). Solubility determination in fasted state-simulating intestinal fluid (FaSSIF) results in modest underestimation of solubility in actual aspirates (37) (Fig. 3). The absence of proteins in FaSSIF might be a possible explanation, since *in vivo* binding to intraluminal proteins may result in increased solubility. Another explanation might be related to the differences in colloidal phases that are present in simulated versus actual intestinal fluids (38).

DRUG SOLUBILITY IN THE FED SMALL INTESTINE

Similar to the fasted state, intrainstestinal drug concentrations in the fed state depend on the water flux across the intestinal wall, the drug concentration in fluids entering the small intestine from the stomach, and the dissolution of particles as they are transferred from the stomach. The hyperosmolarity in the fed small intestine (e.g., 26) is assumed to trigger substantial water secretion. Intrainstestinal dissolution (and therefore solubility) during the digestive phase should be of prime interest for any lipophilic compound. However, as in the fed stomach, solubilizing agents in the fed small intestine may have substantially slower diffusivity than the drug molecules and, therefore, increases of drug solubility may not translate to similar increases in dissolution rates (24).

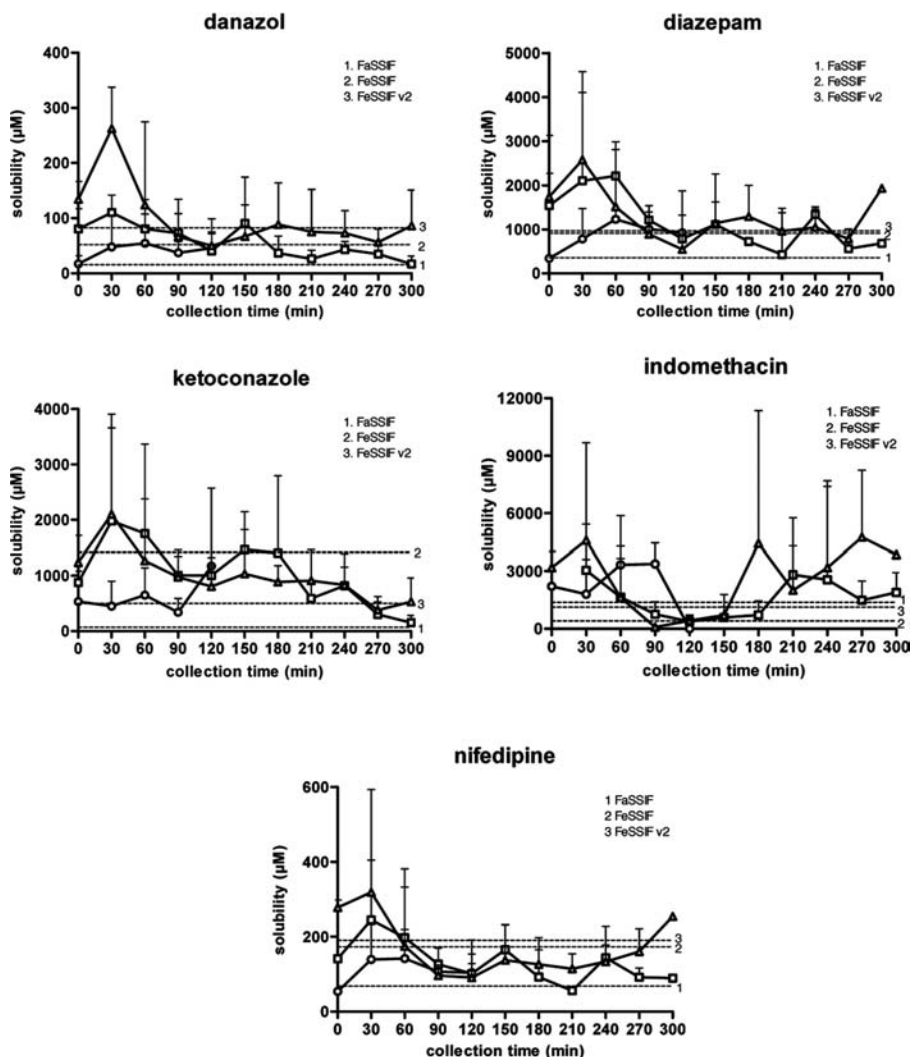


FIGURE 3 Mean ($\pm$ intersubject variability) solubility-time profiles in fasted state ($\circ$ — $\circ$), fed state ($\square$ — $\square$), and fat-enriched fed state (Δ — Δ) HIF for five model drugs. Solubility of the model drugs was assessed in HIF fractions of five subjects unless not enough volume was available ($n \leq 5$). Solubility of the corresponding drug in FaSSIF (pH 6.5), FeSSIF (pH 5.0), and FeSSIF-v2 (pH 5.8) (28) is indicated by broken lines. *Abbreviations:* FeSSIF, fed state—simulating intestinal fluid; FaSSIF, fasted state—simulating intestinal fluid; HIF, human intestinal fluid; FeSSIF-v2, fed state—simulating intestinal fluid version 2. *Source:* From Ref. 38.

Solubility-time profiles in the fed state illustrate a time- and subject-dependent variability, which is greater than that observed in the fasted state (Fig. 3) (24,27,38), in accordance with the changing and variable intraluminal composition (24,27,40). For the compounds tested, the importance of meal's fat

content seems to be minimal (Fig. 3). Limited data, to date (all collected after administration of Ensure plus[®]), do not allow for clear-cut conclusions with regard to the duration of elevated intrainestinal solubility after meal ingestion [reported to vary from 60 minutes (Fig. 3) to more than 180 minutes (27)]. Discrepancies in values reported in the literature are most likely due to differences in the protocol (coadministration of water, total volume administered, and total aspirated volume) (27,38)]. As with FaSSIF, fed state-simulated intestinal fluid (FeSSIF) demonstrates a lower solubilizing capacity compared with human aspirates collected in the fed state after administration of Ensure plus[®] (Fig. 3). The reason relates, at least partly, to the fact that FeSSIF contains no lipid degradation products; the solubilization capacity of FeSSIF-version 2 (FeSSIF-v2) (see chap. 12), which additionally contains oleic acid and monoolein, is higher (Fig. 3). FeSSIF-v2 seems to be a good predictor of intrainestinal solubility of nonionizable drugs, at times longer than 60 minutes (Fig. 3). For the same period, comparisons for ionizable drugs are difficult to make because of the high variability of data in aspirates and the additional effect of pH (Fig. 3). For times up to 60 minutes after meal administration, solubility seems to be increased (Fig. 3) and, therefore, a medium that contains higher concentrations of food lipids needs to be considered (28). On the basis of Figure 4, early-FeSSIF seems to overestimate solubility in aspirates collected 0 to 90 minutes after the meal. In contrast, solubility in middle- and late-FeSSIF was more in line with the solubility reached in human intestinal aspirates collected postprandially after 90 minutes (Fig. 4). Clearly, more data are needed to confirm the above findings.

DRUG SOLUBILITY IN THE COLON

Because of the fact that oral drug absorption is usually complete in the small intestine, the emphasis to date has been given to the conditions in the upper GI lumen. However, in cases where

- the drug has low permeability in the small intestine but can be absorbed to some extent through the colonic mucosa,
- an extended release dosage form is administered, and/or
- the dosage form targets the drug to the colonic region for local action,

conditions in the lower GI lumen will influence drug/dosage form performance. Because of the substantial residence time in the ascending colon (41,42) and the limited free-water volume in the transverse colon (43), the primary region of interest in regard to drug/dosage form performance in the lower gut is the ascending colon. To date, information on the conditions in this region of the intestinal lumen has been very limited and, therefore, in vitro release and/or solubility data have been obtained in media that take into consideration only pH and short-chain fatty acid concentrations measured, for example, in samples from individuals that died suddenly (44). In recent years, the amount of relevant information has increased substantially and more reliable in vitro testing of colon targeting drug/dosage forms is expected in the years to come. For example, volume of contents in the ascending colon is now known to be a few tens of milliliters (43,45), and other physicochemical characteristics that are of prime importance for dosage form performance and/or for drug solubility (e.g., pH, buffer capacity, and osmolality) are different from those observed in the upper intestinal lumen. Interestingly, they are additionally affected by dosing

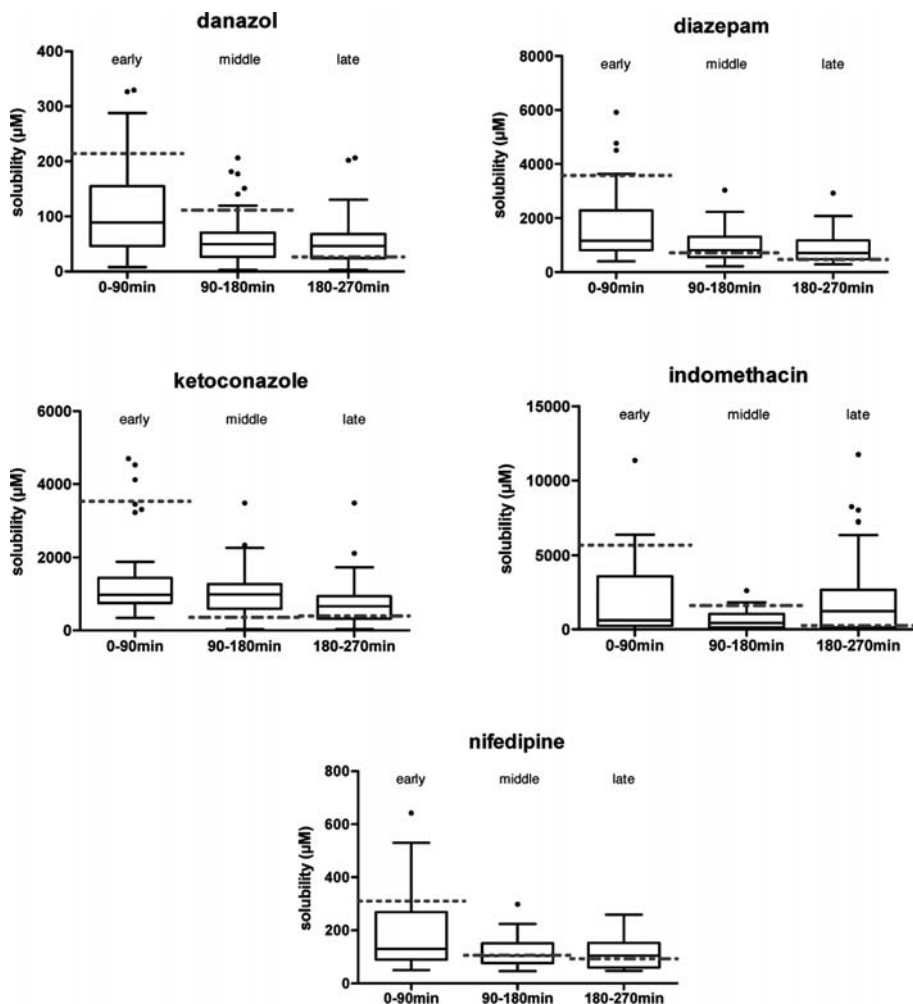


FIGURE 4 Box plots of the solubility of five model drugs in human intestinal fluids aspirated in the fed state and fat-enriched fed state. Results are grouped in early, middle, and late postprandial phases corresponding to the design of early (pH 6.5), middle (pH 5.8), and late FeSSIF (pH 5.4) (28). The solubility in early, middle, and late FeSSIF is indicated by a broken line in the corresponding postprandial phase. *Abbreviation:* FeSSIF, fed state–stimulating intestinal fluid. *Source:* From Ref. 38.

conditions (Fig. 5). Although, in clinical practice the distinction between fasted and fed state conditions is not particularly relevant (most people have residual food in the ascending colon most of the time) in BA/BE studies the delineation between fed and fasted states is more pronounced. Under these testing conditions, differences in buffer capacity, osmolality, and solubilizing agents [i.e., cholesterol, bile acids, phospholipids, and, perhaps, proteins (45)] can affect both drug solubility and drug release, especially from diffusion layer coatings, diffusion matrices, osmotic pumps, and hydrophilic matrices (see chap. 14).

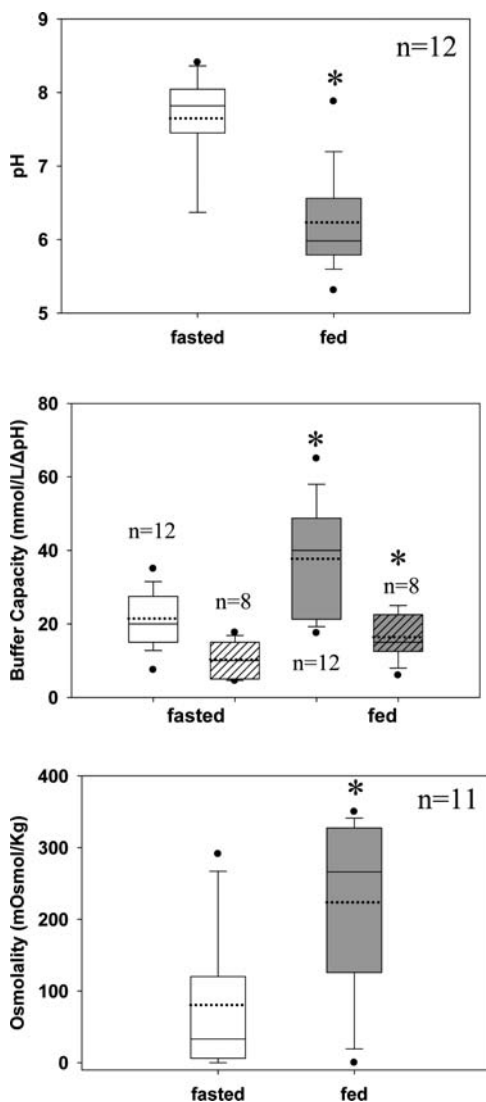


FIGURE 5 pH, buffer capacity [measured with HCl (*plain boxes*) and with NaOH (*lined boxes*)], and osmolality of the contents of ascending colon of healthy adults measured in the fasted state (*white boxes*) and in the fed state (*grey boxes*) on a crossover basis. *n* is the number of subjects contributed to the construction of box plots. For each box plot and from bottom to top continuous horizontal lines indicate the 10th, 25th, 50th (median), 75th and 90th percentile, black dots indicate the individual outlying data points, and the dotted line indicates the mean value. Asterisk (*) indicates that the difference from corresponding fasted state data is statistically significant. Source: From Ref. 45.

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INTRODUCTION

Although major progress has been made in the field of novel drug delivery technologies, oral intake remains the preferred route of drug administration. Oral intake is most convenient for the patient and results in best therapy compliance. Drugs administered orally must exhibit adequate biopharmaceutical properties leading to therapeutic concentrations at the targeted site of action. Early screening of drug candidates, not only for their pharmacological activity, but also for biopharmaceutical and physicochemical properties has become the signature of the contemporary drug discovery paradigm (1).

In addition to possible first-pass elimination by the liver, the rate and extent of intestinal absorption determines the absorptive phase of the plasma concentration-time profile of orally ingested drugs, and thus oral bioavailability. Figure 1 illustrates the various processes defining intestinal drug absorption. To reach the blood circulation, drug molecules should dissolve in the gastrointestinal fluids, upon which they need to overcome the barrier functions of the gastrointestinal mucosa and permeate across the intestinal monolayer of epithelial cells. Incomplete drug absorption may be the result of

1. limited drug release and/or dissolution;
2. degradation, precipitation, or complexation of the drug in the gastrointestinal tract; and
3. limited permeation across the gastrointestinal mucosa.

Both the intraluminal drug concentration (depending on solubility, dissolution and stability in gastrointestinal fluids) and the permeability of the gastrointestinal mucosa for the drug are crucial factors affecting intestinal drug absorption. Estimating the absorption potential of drug candidates during drug development undeniably requires the assessment of these factors.

Permeability assessment during drug discovery and development will be the focus of this chapter. After a brief review of the characteristics of the gastrointestinal barrier, general aspects of permeability measurement will be discussed, while an overview of currently used experimental model systems for assessing drug candidate permeation will be provided. A final section will cover aspects of biorelevance during permeability assessment.

PERMEATION ACROSS THE GASTROINTESTINAL MUCOSA

The gastrointestinal tract is essentially an epithelium-lined channel throughout the body, extending from the mouth to the anus, and presenting an interface between the environment (gastrointestinal lumen) and the body (circulatory

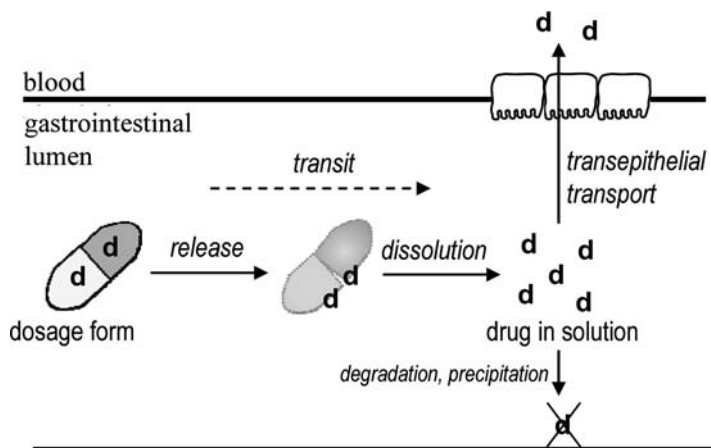


FIGURE 1 Key steps in intestinal drug absorption.

system). It is part of the digestive system and thus specialized in digestion of food, absorption of nutrients, excretion of waste products, and reabsorption of water. The barrier functions of the gastrointestinal wall are instrumental in protecting the body from xenobiotics (2).

The Gastrointestinal Mucosa

The entire gastrointestinal tract is lined with a mucous membrane (mucosa). The mucosa is composed of an epithelial sheet, top-coated with a mucus layer and supported by loose connective tissue (lamina propria) containing both blood and lymphatic capillaries, as well as a thin layer of smooth muscle cells (muscularis mucosae). The precise characteristics of the mucosa vary among different regions of the gastrointestinal tract, depending on their function (Fig. 2). In the

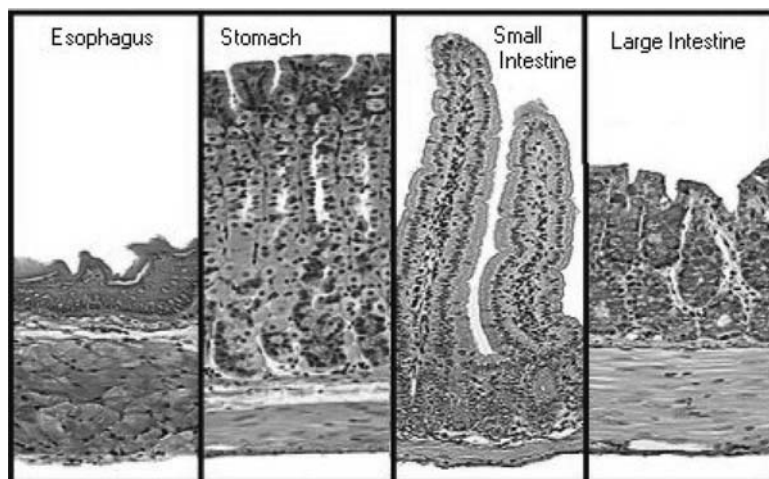


FIGURE 2 Morphological characteristics of the mucosa across the gastrointestinal tract: esophagus, stomach, small intestine, and large intestine, respectively (identical magnification).
Source: From Ref. 3.

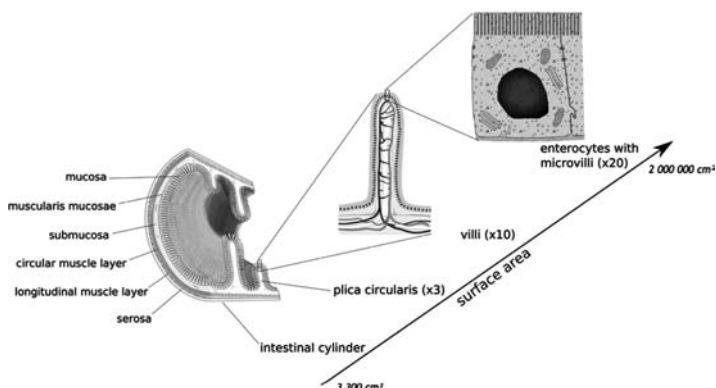


FIGURE 3 Amplification of surface area in the small intestine. *Source:* Adapted from Ref. 2.

absorbing parts of the gastrointestinal tract (stomach, small intestine and part of the large intestine), the mucosa consists of a single layer of epithelial cells (enterocytes) with a richly vascularized lamina propria. These epithelial cells are polarized, having an apical membrane facing the gastrointestinal lumen and a basolateral membrane facing the serosal side. The two membrane domains differ in phospholipid composition and protein expression.

The mucosal surface area is increased through a variety of modifications, including folds, villi (finger-like projections) and microvilli (on the apical cell membrane of the enterocytes). This increase is most pronounced in the small intestine: relative to the surface of a smooth cylinder, the intestinal surface area is enhanced by a factor of 600 (Fig. 3). The enormous surface area of the small intestine (200 m²) compared with that of the stomach (0.053 m²) and the large intestine (0.35 m²) explains the generally higher absorption capacity of the small intestine (4).

The Gastrointestinal Mucosa as a Barrier to Drug Permeation

Physical Barrier

To reach the blood circulation, dissolved drugs have to pass the physical barrier, consisting of a mucus layer and the intestinal monolayer of enterocytes. As mucus (produced by goblet cells) is mainly hydrophilic, it may limit diffusion of strongly lipophilic drugs ($\log P > 3$), resulting in a decreased permeability (5). However, the main barrier for uptake is the intestinal monolayer of epithelial cells. Molecules (both nutrients and xenobiotics) can cross this monolayer via the paracellular (intercellular) or transcellular route (Fig. 4). As the intercellular space occupies less than 0.1% of the total epithelial surface area (6), transport through the paracellular route results in relatively low absorption. Because of the narrowness of the intercellular space formed by the tight junctions between the enterocytes (e.g., 0.8 nm in human jejunum, 0.3 nm in human colon), only small, hydrophilic molecules (e.g., mannitol) will make use of the paracellular route (7). Other molecules will preferably cross the monolayer through the transcellular route (8), following simple passive diffusion based on the existing concentration gradient across the epithelium as the driving force (Fick's law).

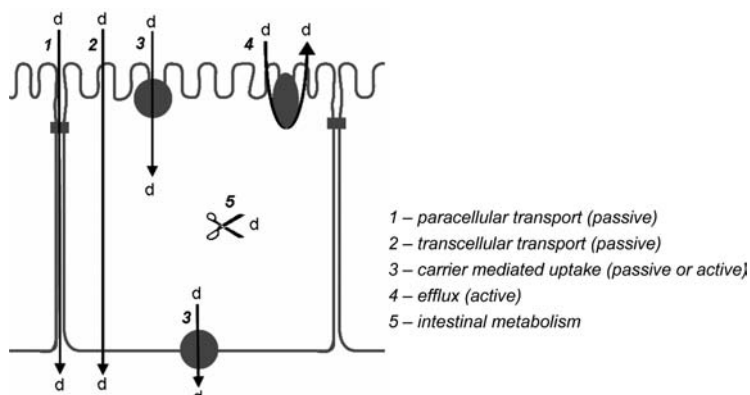


FIGURE 4 Different mechanisms during transport of drug across the intestinal monolayer.

This implies passing two cell membranes (phospholipid bilayers), which may limit diffusion of hydrophilic molecules. In some cases, passive diffusion can be facilitated by the interaction with carrier proteins (transporters) in the cell membrane. In addition to passive diffusion, some molecules can cross the intestinal monolayer by interacting with active transport (nutrient) carriers (e.g. for amino acids or monosaccharides). In this case, the driving force may be hydrolysis of ATP, an electrochemical gradient or co-/countertransport. When carriers are involved, transport is substrate-specific, concentration-dependent, asymmetrical and competitive and may cause drug-drug or drug-food interactions (9).

Biochemical Barrier

Besides being a physical barrier, the intestinal mucosa also presents a biochemical barrier. Key elements of this intestinal biochemical barrier function are intestinal metabolism and efflux (Fig. 4).

In addition to gut microflora and enzymes present in luminal fluids, enzymes in the intestinal mucosa are responsible for intestinal drug metabolism. Although intestinal cytochrome P (CYP)450 enzymes were initially disregarded in terms of importance for phase I metabolism during oral drug absorption (10), more recent findings describe high expression levels of the CYP3A subfamily of enzymes in the mature villus tip enterocytes of the small intestine (11,12). CYP3A4, which represents about 70% of the CYP content in human enterocytes, and CYP3A5, are the major CYPs expressed in human intestine (13). In contrast to CYP3A5, expression levels of intestinal CYP3A4 can be induced by drugs and food components (14,15). Apart from CYP enzymes, esterases, monoamine oxidase and a wide range of hydrolytic and phase II enzymes (e.g., acetyltransferases, glutathione-S-transferases, methyltransferases, sulphotransferases and UDP-glucuronosyltransferases) mediate intestinal drug metabolism (16,17). Further details are described in chapter 4.

Besides metabolism, intestinal efflux may reduce the absorption of various compounds by the active secretion of molecules from epithelial cells into the intestinal lumen. Different efflux transporters classified as ATP-binding cassette

(ABC) proteins, have been identified in the apical membrane of enterocytes. While P-glycoprotein (P-gp, *MDR1*, *ABCB1*) is the most widely studied ABC efflux transporter, BCRP (*ABCG2*) and MRP2 (*ABCC2*) have been demonstrated to be more extensively expressed in human jejunum than P-gp (18). Several reports indicate that the role of intestinal efflux transporters in modulating oral drug absorption is especially important for dual CYP3A/efflux transporter substrates. In such cases, the metabolism and efflux-affinity can severely influence intestinal absorption (19–21). Nevertheless, the in vivo relevance of efflux carriers modulating the oral absorption of drugs remains controversial. This is due to the often-complex interplay between uptake carriers, enzymes and efflux carriers, different expression characteristics in model systems and uncertainty about the functionality of efflux carriers in real intraluminal conditions (see also below). As an illustration, a clinical study with 13 drugs that were all described as substrates for intestinal efflux transporters failed to show significant influence of efflux transport on in vivo drug absorption (22).

Because of metabolism and efflux, the intestine may contribute to pre-systemic elimination (first-pass effect) of xenobiotics. Furthermore, drug-food, drug-excipient and drug-drug interactions that arise from the interaction of intraluminal contents with both enzymes and efflux carriers may increase variability in intestinal absorption (9).

PERMEABILITY MEASUREMENTS

Assessment of intestinal permeability is essential in selecting drug candidates intended for oral administration and to increase insight in the absorption process. This is reflected in the biopharmaceutics classification system (BCS), where permeability is a key parameter to classify drugs according to their biopharmaceutical properties (23). Following the definition of the BCS, drugs are considered highly permeable (classes I and II) when the fraction absorbed in humans is at least 90% (provided the drug is soluble and stable in the gastrointestinal tract). As the fraction absorbed in humans cannot be routinely assessed, especially not for drug candidates, a more practical approach is to determine the intestinal permeability in well characterized model systems and to compare it with the permeability for selected reference compounds (for which the fraction absorbed in humans is known).

Various model systems are available to assess intestinal permeability. Essentially, they differ in the way the gastrointestinal barrier is simulated: artificial membranes, cultured cell layers or real intestinal tissue. Before discussing these model systems in detail, we will provide some general aspects concerning the measurement of permeability.

Permeability Calculations Based on Transport Curves

Irrespective of the model system used, the permeability of a barrier for a drug is assessed by measuring the transport of the drug across the barrier separating a donor and an acceptor compartment. When simulating intestinal absorption, the donor compartment reflects the intestinal lumen, while the acceptor compartment reflects either the intracellular compartment (in the case of a membrane barrier) or the submucosal/serosal side (in case of a cell or tissue-based barrier).

Upon application of a drug in the donor compartment, monitoring the cumulative amount of drug Q transported across a barrier with surface area A into the acceptor compartment in function of time t allows to calculate the flux J .

$$J = \frac{dQ}{dt} \times \frac{1}{A} \quad (1)$$

According to Fick's first law, the flux is in turn proportional to the diffusion coefficient (D) of the penetrating drug molecule and the drug concentration gradient between the donor phase (C_{donor}) and the acceptor phase (C_{acceptor}), divided by the effective thickness of the barrier (h).

$$J = \frac{D}{h} \times (C_{\text{donor}} - C_{\text{acceptor}}) \quad (2)$$

Assuming that the concentration in the acceptor compartment is negligible as compared with the concentration at the donor side (sink conditions) and substituting D/h by the permeability coefficient P , the flux equals

$$J = P \times C_{\text{donor}} \quad (3)$$

By combining equations (1) and (3), the permeability coefficient can thus be calculated as the amount of drug transported per unit of time, corrected for the surface area and donor concentration.

$$P = \frac{dQ}{dt} \times \frac{1}{A \times C_{\text{donor}}} \quad (4)$$

In summary, the assessment of a transport curve (Q in function of t) under sink conditions allows to calculate the permeability coefficient P (typically expressed in cm/sec) by dividing the linear regression slope and transport rate dQ/dt (nmol/sec) by the transport surface area A (cm²) and the donor concentration C_{donor} (μM). Since the donor concentration can be considered constant during the experiment, the initial donor concentration C_0 is used in the calculation.

A typical transport curve is shown in Figure 5. The drug appears in the acceptor compartment after a short period of time (lag time). During this lag

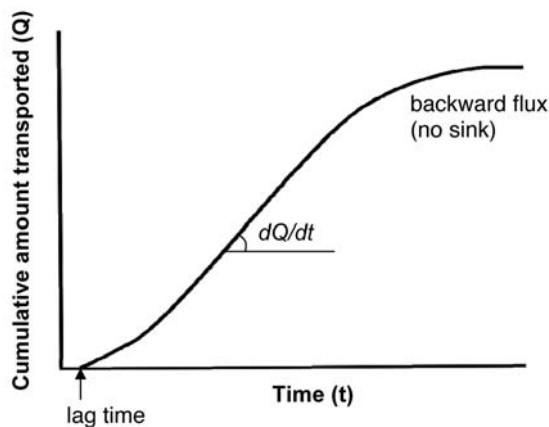


FIGURE 5 Schematic representation of a typical transport curve.

time, the initial transport rate may be limited by intramembrane or intracellular accumulation of the drug. Once these barriers are saturated with drug molecules, a constant transport rate is observed, as long as sink conditions apply. When the drug concentration in the acceptor compartment results in a significant backward flux of drug molecules, net transport will decrease and the assumption of sink condition is no longer valid. Permeability calculations using the above equations should be based on the linear part of the transport curve.

Permeability Calculation Under Nonsink Conditions

Equation (4) is only valid to calculate the permeability coefficient when sink conditions apply. Depending on the rate of transport and the duration of the experiment, significant backward flux may occur and approaches to maintain sink conditions are required (see section “Biorelevance of Basolateral Media: Sink Conditions”). Alternatively, a more general method can be applied to calculate the permeability coefficient, as described by Palm et al. (24). This method is based on Fick’s law taking into account the backward flux.

$$P = \frac{dQ}{dt} \times \frac{1}{A \times (C_{\text{donor}}(t) - Q(t)/V_{\text{acceptor}})} \quad (5)$$

with Q the amount of drug appearing in the acceptor compartment in function of time t , A the surface area of the transport barrier, C_{donor} the drug concentration in the donor compartment in function of time and V_{acceptor} the volume of the acceptor compartment.

Permeability Estimation Based on a Single Time Point

To increase throughput, especially during the early drug discovery phases, permeability is sometimes calculated on the basis of a single measurement of the amount of drug transported into the acceptor compartment (Q) after a certain period of time (t).

$$P = \frac{Q}{t} \times \frac{1}{A \times C_{\text{donor}}} \quad (6)$$

Taking into account the changes in transport rate in function of time (even in the case of a “simple” transport behavior as in Fig. 5), such single time point permeability measurements can only be considered as a rough and not necessarily reliable estimate of the true permeability. Clearly, decisions based on these data should be made with caution, and at the very least the assumption of sink conditions should be verified over the duration of the experiment.

Effective Vs. Apparent Permeability

The endpoint of intestinal drug absorption can be defined as uptake of the drug in the enterocytes or as transport of the drug across the epithelial monolayer. This difference is reflected in the calculation of the effective versus apparent permeability coefficient. The effective permeability coefficient (P_{eff}) refers to transport across the apical membrane (uptake in the enterocytes) while the apparent permeability coefficient (P_{app}) refers to transepithelial transport.

Whether effective or apparent permeability coefficients are measured depends on the type of model system and the experimental setup (see below).

Carrier-Mediated Transport

As stated in section “The Gastrointestinal Mucosa as a Barrier to Drug Permeation” and Figure 4, transport of drugs across the intestinal mucosa can be increased (uptake) or reduced (efflux) by transport proteins in the apical and basolateral membranes of enterocytes. Studying the involvement of these carriers in transepithelial transport is important as they are a potential source of variability in absorption (due to variation in expression/functionality) or mediate interactions of drugs with food or coadministered drugs. A number of approaches are available to investigate carrier functionality.

Assessment of Bidirectional Transport

As a result of the differential expression of uptake and efflux carriers in the apical versus basolateral membrane, the interaction of a drug with such a carrier typically results in a polarity in transport, that is, different rates for absorptive (from apical to basolateral side) versus secretory transport (from basolateral to apical side). A bidirectional transport experiment allows the calculation of a polarity factor (PF) as the ratio of the apparent permeability for the secretory direction versus the apparent permeability in the absorptive direction. Figure 6 illustrates the different possibilities of PF values. A PF = 1 reflects equal transport in both directions, suggesting no interactions with carriers. When PF > 1, secretory transport exceeds absorptive transport, indicating that the drug is a substrate for one or more apically located efflux carriers (in practice PF > 2). When PF < 1, absorptive transport exceeds secretory transport, indicating that the drug is a substrate for uptake carriers (in practice PF < 0.5).

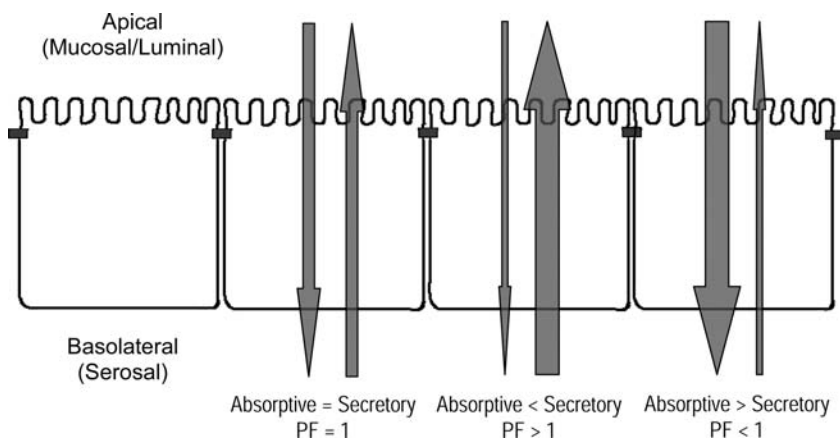


FIGURE 6 Bidirectional transport of a drug across an intestinal monolayer in relation with the polarity factor (PF).

Many drugs are substrates of different uptake/efflux carriers (with potentially opposite effects). Obviously, this complicates the interpretation of PF values. Moreover, care should be taken when interpreting PF values in case of bidirectional transport experiments with weak bases/acids performed in the presence of a pH gradient over the intestinal barrier (see section “pH”). In general, the use of inhibitors is required to evaluate the involvement of specific carriers.

Concentration-Dependent Permeability

When a drug is transported purely by means of passive diffusion, the permeability does not depend on drug concentration. As carriers (and enzymes) can be saturated by increasing the concentration of their substrates, carrier-mediated transport is characterized by concentration-dependent permeability. Saturation of an uptake carrier leads to decreased absorptive permeability and increased secretory permeability. Saturation of an efflux carrier leads to increased absorptive permeability and decreased secretory permeability. In both cases, saturation results in a smaller difference between absorptive and secretory transport (PF closer to 1). Therefore, mechanistic transport experiments should be performed using various donor concentrations.

Use of Inhibitors

The use of carrier inhibitors in (bidirectional) transport studies allows to separate the observed permeability into a passive diffusion and a carrier-mediated component (25). Various inhibitors are available with different potency and specificity, ranging from nonspecific ATPase blockers (e.g., ouabain) to carrier-specific inhibitors (26,27). Using these inhibitors in transport studies provides useful mechanistic information regarding the involvement of carriers (and enzymes) in the transport process. However, the potential cross-reaction of inhibitors with multiple transporters makes it difficult to discern the role of individual transporters (28).

EXPERIMENTAL MODELS FOR PERMEABILITY ASSESSMENT

Various model systems of the intestinal barrier are used to serve different purposes.

1. Screening of drug candidates for their intestinal absorption potential
2. Classifying drugs in the BCS in view of the development of generic formulations
3. Unraveling mechanisms underlying the transepithelial transport of drugs

Table 1 provides an overview of the strengths and limitations of different absorption models that are currently used in academia and industry to measure permeability for drugs and drug candidates. In this section, we will describe the most commonly used membrane-based, cell culture-based and tissue-based (ex vivo and in situ) models and their main applications. The biorelevance of these model systems will be discussed in the final section of this chapter.

TABLE 1 Strengths and Limitations of Currently Used Model Systems for Permeability Assessment

Absorption model	Strengths	Limitations
Artificial membranes (e.g., parallel artificial membrane permeation assay technique)	High throughput Relatively inexpensive Various lipid compositions available Good predictability	Only predictive for transcellular passive uptake Membrane retention of lipophilic compounds Dependent on lipid composition and pH
Caco-2 cell system	Good screening model No bioanalysis (simple salt buffer solutions) Evaluation of transport mechanisms (e.g., polarity in transport) Evaluation of absorption-enhancing strategies on a mechanistic basis Evaluation of toxicity of compounds Reduction of the number of laboratory animals Methods available to increase biorelevance of model Human origin	Lack of mucus-secreting cells resulting in absence of a mucus layer Thickness of UWL is larger than in small intestine Cancer cells, with different or no expression of metabolic enzymes (e.g., absence of cytochrome Ps) "Tighter" monolayer compared with human small intestine (colonic origin) Inter- and intralaboratory variability of permeability data Long differentiation period Relative expression of transporters differs from small intestine Static model
Everted intestinal rings/sacs	Easy and inexpensive to perform Both animal and human tissue can be used Any segment of intestine Useful for mechanistic studies	Viability of tissue (< 30 min) Nonspecific binding and accumulation of lipophilic compounds Suboptimal stirring conditions
Diffusion chambers	Good screening model Good correlation with in vivo permeability data No bioanalysis (simple salt buffer solutions) Possibility to evaluate different regions of the gastrointestinal tract Evaluation of transport mechanisms (e.g., polarity in transport) Evaluation of absorption-enhancing strategies on a mechanistic basis Well-defined absorptive area Good oxygenation	Tissue viability Presence of circular muscle layers during transport studies, resulting in possible underestimation of the permeability Difficulties with UWL Tissue availability (human)
In situ intestinal perfusion	Best simulation of the in vivo situation Evaluation of intestinal absorption without influence of hepatic first-pass metabolism Intact blood flow	Implies anesthesia and surgery Not a screening tool More difficult analysis due to biological media (in case of blood sampling) Laborious and time consuming

Abbreviation: UWL, unstirred water layer.

Source: Adapted from Refs. 29–36.

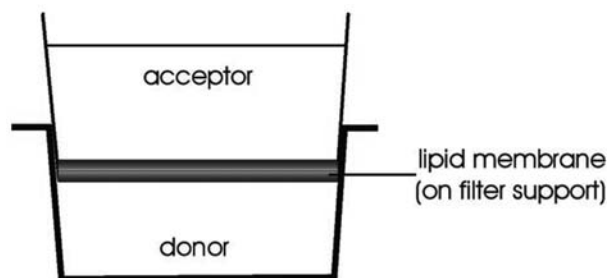


FIGURE 7 Setup of the parallel artificial membrane permeation assay model: transport of drugs across an artificial membrane.

Membrane-Based Models: PAMPA

Description of Parallel Artificial Membrane Permeation Assay

The parallel artificial membrane permeation assay (PAMPA) was introduced to investigate passive permeation processes (37,38). PAMPA implies determining compound permeation across a filter-supported lipid membrane (Fig. 7). The lipid membrane is obtained by adding phospholipids and other membrane constituents, dissolved in an organic solvent, to a hydrophobic filter support. Multilamellar bilayers are expected to form inside the filter channels upon contact with an aqueous medium (37). Several PAMPA setups exist, differing in the lipid composition of the membranes, the supportive filter and the transport media used.

While the original lipid membrane was based on lecithin (containing mainly phosphatidylcholine) (39), other lipid compositions have been evaluated. The phospholipid-free hexadecane PAMPA model (40) provides an easy setup to obtain alkane-water partition coefficients. To increase the relevance of PAMPA in predicting intestinal permeation, a large number of lipid compositions have been evaluated (41–43). A membrane closely resembling the lipid composition of biological membranes (44) is the brush border lipid membrane of Sugano and coworkers (41), consisting of 33% cholesterol, 27% phosphatidylcholine, 27% phosphatidylethanolamine, 7% phosphatidylserine and 7% phosphatidylinositol and having a net negative charge.

The use of hydrophilic instead of hydrophobic filter supports (45) resulted in a significant reduction in transport time to 2 hours (compared with more than 10 hours for a hydrophobic filter). However, depositing lipid mixtures on these filters is more challenging.

Transport media used in PAMPA are based on plain aqueous buffers. As only uncharged molecules permeate across a lipid membrane, transport of ionizable compounds largely depends on the pH used (46,47). While the acceptor medium should be at pH 7.4, the pH of the donor compartment should be varied. Taking into account pH values in the small intestine, Avdeef suggested using two pH values in the donor compartment during screening assays: 6.0 and 7.4 (48,49). To create sink conditions, a sink-forming component (e.g., albumin 3%) may be included in the acceptor medium. The inclusion of solubilizers (e.g., bile acids) in the donor medium may be necessary to solubilize lipophilic molecules and reduce nonspecific adsorption to plastic devices.

Applications of Parallel Artificial Membrane Permeation Assay

Thanks to the relatively easy setup of PAMPA, the system is used in the early phases of drug discovery as high-throughput screening of drug candidates with respect to their ability to permeate across cell membranes (intestinal/blood-brain barrier). For drugs transported purely by passive transcellular diffusion, the measured effective permeability coefficients correlate equally well with human intestinal absorption as compared with permeabilities measured in the Caco-2 system (still considered as the industry standard) (50). Comparison of effective permeability coefficients obtained for drug candidates with those obtained for reference compounds allows classification of drug candidates as high or low permeable.

Screening assays are typically carried out in 96-well format using a sandwich configuration (Fig. 7). Effective permeability calculations are based on a single sampling point. Analyte concentrations are preferably determined by UV absorbance at various wavelengths. However, to improve sensitivity and selectivity, LC-MS has been introduced as an analytical method in PAMPA (51,52).

While PAMPA is favorable for high-throughput screening, it is limited to the measurement of purely passive transcellular diffusion. The potential role of paracellular transport, transport carriers or enzymes cannot be assessed using PAMPA. A combined approach of PAMPA with a more relevant model (e.g., Caco-2) is becoming increasingly popular in drug discovery (47,53).

Cell Culture–Based Models

Description of the Caco-2 System

Compared with artificial membrane-based models, cell culture–based models are more labor-intensive but allow study of different transport mechanisms (para- or transcellular, passive or active). The best-established system is based on Caco-2 cells, originating from a human colon carcinoma (54).

Caco-2 cells spontaneously differentiate into monolayers with most of the morphological, structural and functional characteristics of the intestinal mucosa. Full differentiation requires about 20 days of culture. After this culture period, the polarized cells have formed tight junctions at their lateral interfaces and they express various enzymes, including glutathione S-transferase and some CYP isoenzymes. Additionally, several active uptake carriers (e.g., for peptides, amino acids, glucose, bile acids) and efflux carriers (e.g., MRPs and P-gp) are expressed in differentiated Caco-2 cells. Growing the Caco-2 monolayer on a microporous membrane filter of an insert placed in a well results in a bicompartamental setup with an apical (luminal) and basolateral (serosal) side (Fig. 8).

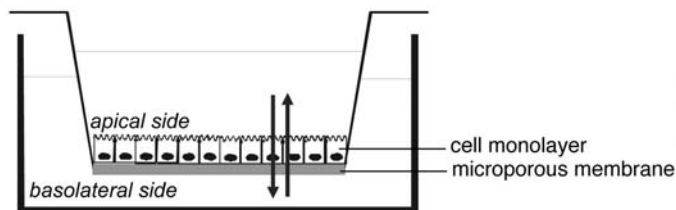


FIGURE 8 Setup of the Caco-2 model system: transport of drugs across a polarized cell monolayer.

This setup allows study of transport in two directions (absorptive and secretory), which is useful for the evaluation of transport mechanisms (e.g., active uptake or efflux).

Despite the similarities between Caco-2 monolayers and the epithelial monolayer *in vivo*, some essential differences need to be considered (see also section "Biorelevance of Model Systems for Permeability Assessment"). In contrast to the *in vivo* situation, the Caco-2 model does not contain mucus-secreting goblet cells; therefore, the impact of mucus on transepithelial transport cannot be assessed. As Caco-2 cells are derived from a colon carcinoma, it is not surprising that the size of the paracellular channels (controlled by tight junctions) is smaller in the Caco-2 model system versus the human small intestine. This may result in an underestimation of paracellular transport (55). Finally, altered expression levels of various enzymes, uptake and efflux transporters have been observed in Caco-2 cells compared with human small and large intestine (17,56–58). For instance, while CYP3A4 and CYP3A5 are the main CYP isoenzymes in human enterocytes, their expression level is extremely low in Caco-2 cells. With respect to efflux carriers, human jejunal enterocytes display higher expression of BCRP than MRP2. In the Caco-2 system, however, the opposite is true (59). In addition, relative transporter expression levels were shown to differ substantially between Caco-2 clones from different laboratories (60).

The use of cell monolayers for permeability assessment requires the evaluation of the integrity of the monolayer before and after the transport study. Two common approaches include measuring the transepithelial electrical resistance (TEER) and monitoring the flux of a hydrophilic marker molecule that passes the monolayer by the paracellular route (e.g., atenolol, mannitol, sodium fluorescein). Figure 9 clearly shows that a decrease in TEER ($\leq 80\%$ of the initial value) during transport experiments in the Caco-2 system results in an increase of the flux of the paracellular marker sodium fluorescein.

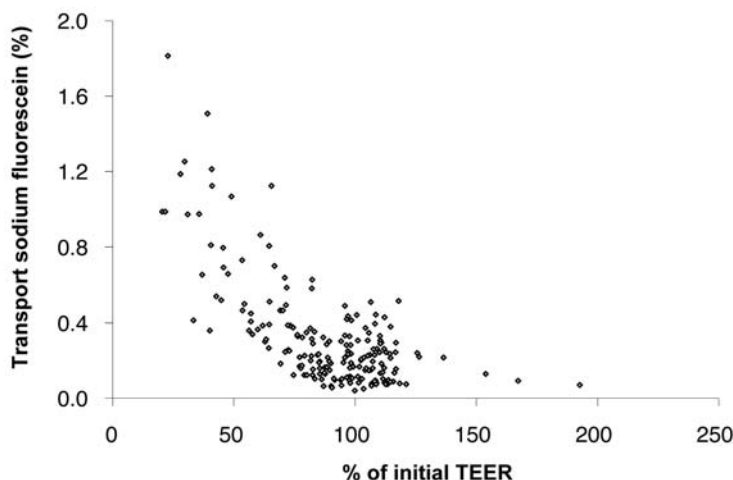


FIGURE 9 The relation between the transport of sodium fluorescein and transepithelial electrical resistance (TEER) values at the end of the experiment in the Caco-2 model system.

Applications of the Caco-2 Model

Screening of drug candidates. The Caco-2 system can be considered as the industry standard for screening drug candidates with respect to permeability across an intestinal monolayer. The observed sigmoidal relation between Caco-2 apparent permeability and fraction absorbed in humans allows the identification of drug candidates that are likely to suffer from limited transepithelial transport in vivo (8). These correlations are excellent for compounds transported via transcellular passive diffusion, but less reliable for compounds transported via paracellular diffusion (potential underestimation in Caco-2) or for compounds that interact with transporters or enzymes (due to different expression levels).

The use of a model system for high-throughput screening implies specific challenges. In this respect, a lot of effort has been made to automate and miniaturize the Caco-2 model system (61). In addition, various attempts have been made to reduce the required culturing time by altering the filter support, the filter coating, the seeding density and the cell culture medium (61–64). Ranking passively transported compounds with respect to permeability may be possible with Caco-2 monolayers cultured for a short period of time (e.g., three days); however, these monolayers are not suitable for mechanistic and bidirectional studies, as the expression of transporters is significantly lower (62). Finally, care should be taken to avoid that the analysis of the generated samples becomes the bottleneck for the whole experiment. In this respect, a compromise between the speed of a UV plate reader and the selectivity and sensitivity of LC/MS is often required. The inclusion of additives in the transport medium (see section “Biorelevance of Basolateral Media: Sink Conditions”) may further complicate and slow down the analytical procedure.

The very low solubility observed for many new drug candidates often impedes determining the absorption potential in in vitro models. The multitude of approaches that have been used to increase solubility and thereby the reliability of permeability measurement (including cosolvents and solubilizing excipients) has been reviewed by Ingels and Augustijns (65).

Mechanistic studies. Caco-2 monolayers enable study of a variety of transepithelial drug transport mechanisms, including passive transcellular and paracellular diffusion, active uptake and efflux, and metabolism. A list of examples of the use of the Caco-2 system to explore the mechanisms behind drug-drug, drug-food and drug-excipient interactions, and to evaluate strategies for enhanced oral absorption, can be found in Table 2.

Standardization of the Caco-2 Model

Interlaboratory comparisons between Caco-2 permeabilities for the same compounds reveal significant differences (8,60). These differences are due to variations in culturing conditions, experimental conditions, and age of the cells (passage number and culture duration) (93–95). For instance, the expression level of transport proteins is known to vary significantly with the age of cell cultures (56,58,94,96).

In addition to standardization of cell culture procedures and protocols across all laboratories, the use of a set of internal reference compounds as controls is required (65). Such a set should comprise compounds with different transport characteristics (high, low and zero permeability, passive and active

TABLE 2 Examples of Mechanistic Transport Studies in the Caco-2 Model System

Purpose	Examples (references)
Role of transporters in drug absorption	
Drug-drug interactions	Interaction between P-gp substrates and inhibitors (13,66–68) Interaction between MRP1, MRP2, BCRP substrates, and inhibitors (69–73)
Drug-food interactions	Inhibition of P-gp efflux by fruit extracts (74,75)
Drug-excipient interactions	Effect of nonionic surfactants on membrane transporters (76–79)
Functionality of efflux carriers in biorelevant conditions	Decreased functionality of P-gp in presence of human or simulated intestinal fluid (80–82)
Evaluation of absorption-enhancing strategies	
Absorption enhancers	Effect of excipients on paracellular transport of low-permeability compounds (83,84)
Lipid-based strategies	Increased absorption of low-solubility drugs (85,86)
Prodrug strategies	
For low-permeability compounds	Enhanced absorption of esterase-sensitive prodrugs (87–89)
For low-solubility compounds	Enhanced flux of poorly soluble drugs from phosphate ester prodrugs (90,91)
Supersaturation	Enhanced flux of itraconazole from supersaturated solutions (92)

transport) (see section “Permeability for Marker Compounds in Different Model Systems”). When using permeability data from Caco-2 monolayers or another in vitro model system for applying biowaivers, the FDA provides a list of possible internal standards in its “Guidance for industry: waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system” (97).

Other Cell Culture–Based Model Systems

While the Caco-2 system is the most commonly used cell culture–based model for drug transport, other cell lines might be utilized for specific purposes (98).

Madin-Darby canine kidney (MDCK) cells can be considered as an alternative to Caco-2 cells for permeability screening (99,100). Like Caco-2 cells, MDCK cells spontaneously develop tight junctions and form monolayers of polarized cells. Full differentiation requires only 3 to 5 days versus 20 days for Caco-2 cells. Another advantage of MDCK cells are the lower TEER values and increased paracellular transport of hydrophilic transport which probably resembles the in vivo situation of human intestinal mucosa more closely. A disadvantage, however, is the nonhuman (canine) and nonintestinal (renal) origin of MDCK cells.

The MDCK and LLC-PK1 (derived from pig kidney epithelial cells) cell lines are both polarized cells with low expression levels of transport proteins and low metabolic activity (101). The fact that these cells can be transfected relatively easily makes them an interesting option for mechanistic studies that aim to study the specific effect of a single transporter on drug permeability (102).

For instance, measuring drug transport across MDCK cells that are stably transfected with P-gp/MDR1 as compared with parent MDCK cells can be used to selectively evaluate the influence of P-gp on drug transport (103).

Other, less commonly used cell lines include the following:

1. The rat duodenal cell line 2/4/A1, which might be more relevant than Caco-2 cells to study paracellular transport (55)
2. The TC7 cell line, which is a Caco-2 subclone with an increased expression of CYP3A4 and CYP3A5 and which may be used to evaluate the role of metabolism in transepithelial transport (104)
3. The HT29-MTX model which comprises a coculture of human-derived enterocytes and mucus producing goblet cells (105)

Everted Intestinal Rings/Sacs

Description

Everted intestinal sacs/rings are a relatively simple system for absorption measurement. In this method, a section of the intestine is isolated immediately after euthanizing the animal and washed in ice-cold buffer to remove debris and digestive products. One end of the cut intestinal section is tied with a piece of suture and the closed end is carefully pushed through the intestine using a glass rod, resulting in an inside-out intestinal segment.

To obtain intestinal rings, the tissue is cut into 2 to 4 mm wide rings (106). The rings are incubated in a carbogen oxygenated buffer solution containing the compound under investigation and shaken well in a water bath. After a designated time interval, rings are taken out of the solution, blotted dry, weighed and dissolved or processed for analysis. The uptake of the compound can be measured by radiolabel counting or fluorescence assay.

In contrast to the intestinal rings, only the mucosa is in contact with the permeant in the intestinal sac model. The sac is filled with buffer and put in a flask with carbogen-oxygenated buffer containing the compound under investigation. At the end of the experiment, the sac is opened at one end and the serosal fluid is collected (107). Integrity of the tissue during the experiment can be monitored by measuring the transport of a marker such as trypan blue dye.

Applications of Everted Intestinal Rings/Sacs

Despite its simplicity, the use of intestinal rings/sacs for permeability assessment is relatively rare. Under appropriate conditions, the *in vitro* uptake of a series of drugs into the rings closely parallels the known *in vivo* absorption of these drugs (108). Moreover, the uptake is relatively independent of tissue origin and cosolvent. The latter was demonstrated in a paper showing the application of 1-methyl-pyrrolidine as a cosolvent in transport measurements of poorly soluble compounds (109).

With the method of everted intestinal rings, both passive processes and carrier-mediated transport have been demonstrated (108,110–114). A good correlation with *in vivo* absorption was reported for a set of 11 structurally unrelated compounds, including both passively and actively transported compounds, ranging from extremely low to very high bioavailability (108). Everted intestinal rings can also be used to study differences in permeability between various intestinal regions (115).

In contrast to everted intestinal rings, para- and transcellular diffusion can be discriminated with the everted intestinal sac model (116). Applying a slight modification to the model, Tomita and coworkers showed the influence of absorption promoters such as sodium caprate and laurate, on the paracellular permeation of cefmetazole and inulin (116). As the serosal volume is low compared with the area for absorption, everted intestinal sacs can be used to perform absorption experiments for low-solubility compounds or low concentrations of drugs. Everted intestinal sacs have also been applied for the investigation of drug metabolism (117).

Limitations of Everted Intestinal Rings/Sacs

Although the model of everted intestinal rings has several advantages, including its simple use and the fact that an impressive set of rings can be prepared from one piece of intestine, this model also has its limitations. The transport of the solute into the rings includes all areas accessed by the incubation solution, not only through the luminal membrane; connective tissue and muscle tissue are also exposed to drug solution and included in the calculation of uptake (109). Furthermore, the paracellular and the transcellular transport route cannot be distinguished with this method. Viability of the everted segments might be an issue. Everted intestinal ring segments are claimed to be viable for a period of only 30 to 60 minutes, even when they are maintained before use in a physiological buffer containing glucose (108). Also the method of sacrifice and anesthesia used seems to play a significant role in maintaining the viability of the tissue during the experiment (118).

Similar to the everted intestinal rings, the everted intestinal sac model is an inexpensive technique that is relatively simple and allows several experiments to be performed using tissue from just one intestine. This model can be a useful tool for studying mechanistic aspects of absorption, especially for assessing absorption from different parts of the small intestine and colon. However, the nonspecific binding, the suboptimal stirring conditions and the short viability of the intestinal segments remain serious limitations of this method.

Ex Vivo Models (Diffusion Chambers)

Description

Excised intestinal segments, obtained from anesthetized animals (or sometimes from human surgical waste) and mounted between two diffusion cell compartments, were initially used to investigate ion transport related to electrophysiological phenomena (119). The model was later adapted by Grass and Sweetana to study intestinal drug transport (120). Inclusion of the test compound to either the mucosal or serosal side of the tissue allows determination of absorptive and secretory permeability coefficients (29). A schematic representation of a typical diffusion chamber setup is shown in Figure 10.

Obviously, the viability and integrity of the excised intestinal segments are critical for the reliability of transport data. The use of specialized transport media [i.e., carbogen (O₂:CO₂ 95:5)-gassed Krebs-Ringer bicarbonate buffer, sometimes supplemented with glucose, glutamate, fumarate and pyruvate (121)] helps to maintain tissue viability and integrity. However, intestinal edema and disruption of the villi may occur after as little as 20 minutes of incubation (122). Also edge damage of the excised tissue may result in loss of tissue integrity (123,124).

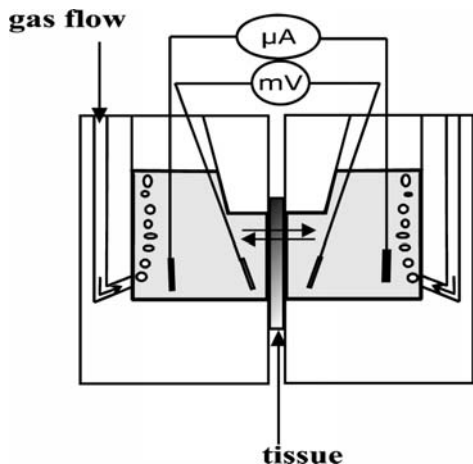


FIGURE 10 Setup of the diffusion chamber model: transport of drugs across excised intestinal tissue.

Therefore, it is critical to monitor tissue viability and integrity during the course of transport experiments. Electrophysiological parameters, including the potential difference (PD), the transepithelial resistance (TEER or R_T) across the tissue and the short circuit current (SCC or I_{SC}) reflecting ionic fluxes across the epithelium, are considered to reflect tissue viability and integrity (125). For instance, a time-dependent increase in permeability of excised intestinal tissue for propranolol (transcellular transport) and mannitol (paracellular transport) was related to an evolution in electrical parameters (125). As an alternative to monitoring electrophysiological parameters, marker molecules for paracellular transport, such as mannitol, inulin, Na-fluorescein and PEG-400 have been used to verify the integrity of the epithelial layer (30). On the basis of tissue viability data, extremes in permeability values can be discarded from the data set, resulting in more consistent transport data (121,125–128). As for all permeability models (see section “Standardization of the Caco-2 Model”), it is advisable to use a set of reference compounds to verify the functionality of the main transport routes (see section “Permeability for Marker Compounds in Different Model Systems”).

Applications of Diffusion Chambers

Clearly, diffusion chambers cannot be implemented as a high-throughput screening tool, but they are suited for several types of mechanistic studies. Given the physiologically relevant expression levels of transporters and enzymes in *ex vivo* intestinal tissue, the diffusion chamber technique is often more predictive than cell culture-based models for studying the effect of intestinal efflux (121,129–132) and metabolism (87,133,134) on the absorption process. Also the dynamic interplay between CYP3A and P-gp in intestinal drug disposition has been explored using the diffusion chamber approach (135). The influence of intraluminal components (e.g., fruit extracts, surfactants, excipients) on tissue integrity and drug permeation has been investigated (130,131,136,137); however, it should be noted that the continuous gassing of the diffusion chambers with carbogen complicates the addition of surfactants and proteins in high concentrations due to foaming.

The use of intestinal tissue enables certain studies to be performed that are not possible with cell culture-based models. For instance, the diffusion chamber technique offers the ability to study regional differences in intestinal absorption throughout the whole intestinal tract (123,127,138–141). Furthermore, inter-species differences in intestinal absorption can be determined, which can be useful for the selection of an adequate animal model for bioavailability and pharmacokinetic studies (139,142). To investigate the impact of transporters, transport across tissue from mutant versus wild-type animals can be compared. For instance, Mallants et al. used intestinal segments from MRP2-deficient rats to study the role of MRP2 in intestinal efflux of tenofovir disoproxil fumarate (143).

In general, data obtained in diffusion chambers are closely related to the in vivo situation (30,144). Diffusion chambers using rat intestinal segments also appeared to be a useful method to classify compounds with high and low permeability according to the BCS (127).

In Situ Intestinal Perfusion Model

Description

Since its first introduction by Schanker and coworkers (145), the intestinal perfusion model has proven to be a powerful research tool for the investigation of intestinal transport and metabolism. In this model, an animal (generally a rat) is anesthetized, and placed on a heating pad to maintain constant body temperature, after which a laparotomy is performed. Consequently, two L-shaped cannulas are inserted at the duodenal and ileal end of the isolated intestine and the intestinal content is removed by purging the intestine with perfusion medium.

In the closed loop setup, which was originally described by Doluisio et al. (146), the drug solution is continuously circulated in a closed loop through the intestine during a fixed time period. At predefined time points, samples are taken from the drug solution to evaluate the amount of compound that has disappeared from the medium. In the single-pass intestinal perfusion model (Fig. 11), the drug solution is perfused continuously down a set length of intestine through the duodenal end cannula and the perfusate is collected from the ileal end (147). The samples collected at outflow are then analyzed and the concentration difference between inlet and outlet fluids at steady state is determined.

In both setups the effective permeability coefficient (P_{eff}) is calculated on the basis of the disappearance of the drug from the perfusate (148).

$$P_{\text{eff}} = F \times \frac{\left(1 - \frac{C_{\text{out}}}{C_{\text{in}}}\right)}{2\pi RL} \quad (7)$$

with F the flow rate of the perfusate, C_{out} and C_{in} the outlet and inlet concentrations, respectively, and R and L the radius and length of the perfused intestinal segment, respectively ($R \cong 0.2$ cm in rat).

In both the closed loop and single-pass setup, drug absorption is predicted by quantifying net drug uptake into enterocytes (effective permeability coefficient) rather than net flux through the cell. By applying a plasma sampling technique at the mesenteric vein (149), the apparent permeability coefficient (P_{app})

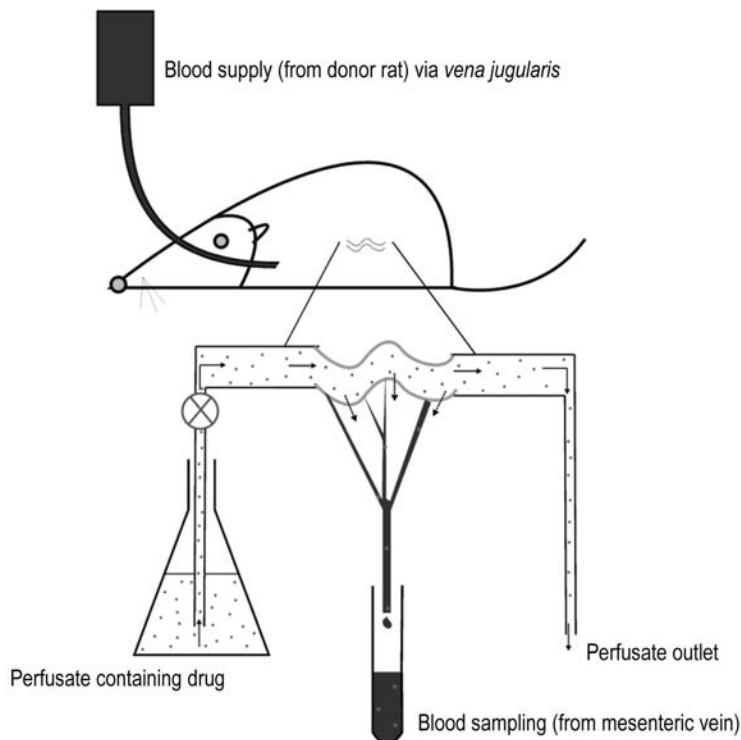


FIGURE 11 Setup of the in situ intestinal perfusion model (single-pass setup with blood sampling).

can be quantified on the basis of appearance kinetics in prehepatic blood and the clearance Cl of the drug from the perfusate (150).

$$P_{app} = \frac{Cl}{2\pi RL} \quad (8)$$

and

$$Cl = \frac{Q_t}{AUC_{0-t}} \quad (9)$$

with Q_t the cumulative amount of drug and metabolites absorbed in the blood at time t and AUC_{0-t} the area under the curve of the perfusate concentration-time profile. If the perfusate concentration can be considered as constant, equation (4) can also be used (with dQ/dt the appearance rate of the drug in the blood and $A = 2\pi RL$).

Applications of In Situ Model

The in situ perfusion model offers the best simulation of the in vivo situation (e.g., presence of mucus, relevant barrier functions, sink conditions, etc.).

However, the influence of the anesthesia, the surgical procedures and the perfusion rate on the outcome of the experiment is not always clear. As the in situ intestinal perfusion model is time consuming and relies on animals, it can clearly not be used as a (high-throughput) screening model.

The primary application of the in situ intestinal perfusion model is to predict absorption of both passive and carrier-mediated substances (151–153). In general, good correlations with effective permeability determined in human jejunum are obtained (144).

The model has also been used to evaluate absorption-enhancing strategies. For instance, the potentially beneficial effects of inhibiting efflux or intracellular metabolism have been investigated (149,154). Recently, the potential of a supersaturation-inducing formulation to increase the absorption of the poorly water-soluble drug itraconazole was illustrated using the in situ perfusion model (92).

Similar to the diffusion chamber technique, the in situ perfusion technique can be used to compare permeability differences between regions along the intestine, which is a prerequisite for the evaluation of controlled-release products (127). Moreover, the use of mutant rats (143) or even knock-out mice, deficient in the expression of, for example, transport carriers, may increase insight in the role of specific carriers in transepithelial drug transport.

Drug Disappearance from the Intestinal Lumen as Measure of Drug Absorption

The main drawback of the intestinal perfusion model without mesenteric blood sampling is the assumption that drug disappearance reflects drug absorption in the calculation of the effective permeability coefficient. This assumption is not valid when the compound suffers from nonspecific binding to the tubing or the gut wall. Nonspecific binding to tubing may be reduced by addition of solubilizing agents or bile acids to the medium or by using coated tubing. Moreover, intestinal perfusion models based on disappearance kinetics assume that drug transport into the enterocyte (through the apical membrane) is rate limiting to the overall absorption (155). This view is most likely true in the case of passively absorbed, stable compounds. However, if the studied compound is metabolized by or accumulates in enterocytes, drug disappearance from the perfusion solution will not reflect drug appearance into the blood. For compounds that are actively taken up, passage of the basolateral membrane may be the rate-limiting step in the overall transport from lumen to portal blood. For compounds that are transported via intestinal lymphatics (e.g., highly lipophilic drugs), association with intracellularly produced lipoproteins appears to be the critical step for access to the systemic circulation (156).

As mentioned before, sampling mesenteric blood circumvents the limitations of the disappearance kinetics model. However, because of the presence of the biological matrix, the analysis of blood/plasma samples is far more complex than the analysis of aqueous buffer solutions.

Permeability for Marker Compounds in Different Model Systems

Comparison of the characteristics and features of the available model systems for permeability measurement reveals substantial differences in barrier functions. In addition, discrepancies in cell culture, tissue manipulation and experimental

TABLE 3 Absorptive Permeability for Marker Compounds Atenolol, Propranolol, and Talinolol in Different Model Systems

	Absorptive permeability P (10^{-6} cm/sec)		
	Paracellular	Transcellular	Transcellular, P-gp
	Atenolol	Propranolol	Talinolol
Parallel artificial membrane permeation assay (P_{eff})	0.052 ± 0.004	22.6 ± 1.4	Not determined
Caco-2 (P_{app})	0.27 ± 0.01	23.5 ± 0.4	0.69 ± 0.09
Diffusion chambers (rat ileum, P_{app})	5.9 ± 1.8	21.2 ± 5.7	1.9 ± 0.6
In situ perfusion (rat ileum, P_{app})	1.6 ± 0.3	38.6 ± 17.8	1.2 ± 0.1

conditions may result in interlaboratory or interbatch variability in permeability assessment. To improve the quality of permeability data, a set of marker compounds can be used as reference to verify the functionality of the different barriers. For instance, the following mixture of β -blockers includes a marker for paracellular transport (atenolol), a marker for transcellular transport (propranolol) and a marker for P-gp mediated efflux (talinolol). An analytical method to simultaneously determine these compounds has been described (157).

Table 3 reports the absorptive permeabilities for these marker compounds measured in four commonly used model systems for permeability assessment: PAMPA, Caco-2, diffusion chambers with rat intestinal tissue and in situ perfusion of rat intestine (using aqueous buffers at pH 7.4 as transport medium). For the compounds transported by purely passive diffusion, the permeability for propranolol (transcellular) is significantly higher than the permeability for atenolol (paracellular) in the various model systems. This reflects the limited contribution of the paracellular route to transepithelial transport. The difference is most pronounced in the PAMPA system, which does not feature the paracellular route, and in the Caco-2 model originating from colonic tissue, which has a tighter monolayer. The absorptive permeability of both Caco-2 monolayers and rat intestinal tissue for the P-gp substrate talinolol is much lower than for propranolol. Comparing the absorptive transport of talinolol with the secretory transport in the Caco-2 system ($P_{\text{app, secr}} = 13.7 \pm 0.4 \cdot 10^{-6}$ cm/sec) and the diffusion chambers ($P_{\text{app, secr}} = 13.4 \pm 0.7 \cdot 10^{-6}$ cm/sec) reveals a PF of more than 7; this PF is reduced in the presence of the P-gp inhibitor verapamil. In the in situ perfusion model, the absorptive permeability for talinolol is increased upon inclusion of verapamil in the perfusate ($P_{\text{app}} = 4.8 \pm 0.7 \cdot 10^{-6}$ cm/sec). These observations clearly indicate that the efflux carrier P-gp attenuates the intestinal uptake of talinolol.

ISSUES RELATED TO THE BIORELEVANCE OF PERMEABILITY ASSESSMENT

As preclinical model systems for permeability assessment are used to take decisions concerning intestinal absorption in humans, they should adequately represent the in vivo situation. In this section, we provide an overview of some common issues related to the biorelevance of the approaches discussed in section "Experimental Models for Permeability Assessment."

Biorelevance of Model Systems for Permeability Assessment

(Non)adequate Modeling of In Vivo Barriers

As stated in section "The Gastrointestinal Mucosa as a Barrier to Drug Permeation," drug molecules have to pass a number of barriers before they reach the blood circulation, including physical barriers (mucus and a monolayer of enterocytes) as well as biochemical barriers (carriers and enzymes). It is obvious that no model system for permeability assessment simulates these barrier functions in the human small intestine to perfection.

Mucus. The hydrophilic and negatively charged mucus layer at the apical side of the enterocytes may significantly retard transport on the basis of charge, size, and lipophilicity (5). However, a mucus layer is not present in (artificial) membrane-based or in most cell culture-based models. The potential role of the mucus layer on permeability assessment can only be modeled using a specialized cell culture-based model (HT29-MTX), tissue-based models or the in situ perfusion technique. In tissue-based systems it is unclear to what extent the mucus layer is affected by tissue manipulation.

Intestinal monolayer and biochemical barrier functions. Membrane-based models do not contain a monolayer of cells and can therefore not be used to study paracellular or carrier-mediated transport. The question as to what extent this limitation hampers the usefulness of PAMPA in drug discovery is still under discussion. Recently, Galinis-Luciani and coworkers (158) critically evaluated the use of PAMPA in comparison with other high-throughput screening tools including octanol/water partitioning and calculated log D values. They concluded that PAMPA did not provide additional information in the selection of drug candidates as compared with calculated log D values. In response to this publication, Avdeef and colleagues (50) provided data to show that PAMPA has a greater predictive value for oral absorption than the octanol/water partition coefficient for real-world drug discovery compounds and that the assay conditions are critical to generate high-quality data.

In cell culture-based model systems, the properties of the intestinal monolayer depend on the origin of the cell line and the culturing conditions. Differences in the nature of tight junctions may affect paracellular transport. For instance, the rat intestinal cell line 2/4/A1 mimics the paracellular pore size radius of the human small intestine better than colon-derived Caco-2 monolayers (9 vs. 4 Å, respectively). As a result, the permeability for various poorly permeable drugs was up to 300-fold higher across 2/4/A1 versus Caco-2 monolayers, thereby better simulating the permeability across human tissue (55). The expression patterns of enzymes and transporters in cell culture-based models may strongly differ from the human small intestine (18,56–58). Clearly, data concerning biochemical barrier functions in these model systems cannot simply be extrapolated to the in vivo situation, but may direct further research. Moreover, correct interpretation of this type of data is often complicated by the lack of characterization of the model in use (98).

In contrast to cell culture-based models, animal tissues (used ex vivo or in situ) contain enzymes and transporters at their normal in vivo expression levels, although their functionality can be altered by the experimental conditions. Obviously, interspecies differences may complicate extrapolation to

humans. Lennernäs (144) reviewed the correlation between the permeability of rat and human intestinal tissue. Comparing *in situ* perfusion in rat small intestine with *in vivo* perfusion in human jejunum, a strong correlation between the respective effective permeability coefficients has been observed for drugs transported by both passive and active mechanisms, although some actively absorbed compounds (e.g., L-dopa and glucose) do not fit in this correlation. The effective permeability is on average threefold higher in humans than in rats. With respect to the expression levels of transporters and enzymes in rat and human small intestine, a reasonable correlation has been reported for transporters, but not for enzymes. Therefore, the impact of intracellular metabolism on drug absorption may strongly differ between rat and human (159). It should be noted that, when measuring effective permeability coefficients on the basis of disappearance of drug from the lumen, metabolism in the enterocytes is not taken into account (see section “Drug Disappearance from the Intestinal Lumen as Measure of Drug Absorption”).

Nonrelevant Barriers for Permeation in Model Systems

Drug molecules may encounter additional barriers in permeability models that are not biorelevant. Obviously, they should be taken into account when extrapolating data to the *in vivo* situation.

Unstirred water layer. The unstirred water layer (UWL) or aqueous boundary layer is an aqueous layer adjacent to biological membranes. Depending on the thickness of the UWL, this layer can be a diffusion barrier (rate-limiting step) for highly permeable drugs (strong lipophilic and/or actively transported). *In vivo*, the UWL is relatively thin (30–100 μm) because of the motility of the gastrointestinal wall (155). Therefore, its role in controlling the intestinal absorption *in vivo* is probably quite small. However, the reduced motility in experimental models of intestinal absorption, including PAMPA and cell culture-based models, results in increased thickness of the UWL. The thickness of the UWL in unstirred PAMPA has been estimated to be 1900 to 3800 μm (160). Although shaking the PAMPA setup reduced the thickness of the UWL (160), individual-well magnetic stirring was required to reduce the UWL to the *in vivo* range. This not only significantly reduced the assay time (from 15 hours to 15 minutes) but also increased the effective permeability of lipophilic compounds (161). The effect of the UWL on drug transport was also demonstrated in the Caco-2 model (162).

In the diffusion chambers model [see section “Ex Vivo Models (Diffusion Chambers)”], the fluid circulation resulting from the continuous gassing of the medium is expected to sufficiently reduce the thickness of the UWL (120).

Nonspecific adsorption, membrane retention, and tissue accumulation. Calculating a mass balance after performing a transport experiment often reveals a low recovery of the drug, especially for lipophilic compounds. Assuming the drug is not degraded, this poor recovery can be due to nonspecific adsorption to plastic devices or filters, membrane retention and/or intracellular accumulation. While adsorption to plastic devices is clearly not biorelevant, it is unclear to what extent membrane retention and intracellular accumulation also occur *in vivo*. It is often assumed that these events are less pronounced *in vivo*, as a result of

optimal sink conditions (drug molecules are carried away by the blood circulation). Together, these phenomena can be considered as an additional barrier to permeation. Physical loss of the compound results in a reduced concentration in the donor compartment (driving force), and thus in an underestimation of permeability. When selecting drug candidates, this may result in false negatives. A mass balance is definitely required to correctly interpret the outcome of the experiments.

The impact of membrane retention has been evaluated in PAMPA (42,163,164). One can correct for membrane retention by calculating the mass of the compound lost in membranes from the difference between the total starting amount and the amounts in donor and receiver compartments at the end of the experiment (165). However, this approach does not take into account other factors that result in a decreased recovery, including degradation of the drug and nonspecific adsorption to plastic devices or filters.

To minimize nonspecific adsorption, a few approaches have been suggested for both PAMPA and the Caco-2 model. The inclusion of surface-active agents (e.g., bile acids or cyclodextrins) or cosolvents (38,166,167) in the donor compartment may not only reduce nonspecific binding but may also increase the solubility of lipophilic drugs and enhance the accuracy of the permeability assay (65). However, these additives may have multiple additional effects, for example, reduction of the free fraction of drug, alterations of the barrier function, etc. An additional postexperimental step of washing the receiver compartment with organic solvents can also increase the recovery (168), although it might not be suitable for high throughput. Another approach to the adsorption issue involves the addition of serum proteins (169,170) or micelle-forming excipients, such as Gelucire 44/14, Cremophor EL or TPGS (171) to the receiver compartment, leading to an improved assay recovery and better predictability of the model (65).

In the case of excised intestinal tissue mounted in diffusion chambers [see section "Ex Vivo Models (Diffusion Chambers)"], molecules have to pass not only the intestinal mucosa but also the circular and longitudinal muscle layer to reach the receiver compartment (172). These muscle layers are an additional barrier for transport and drugs may accumulate in the muscle layer. A partial solution is to strip the longitudinal muscle layer from the intestinal tissue; the circular muscle layer cannot be removed without damaging the intestinal monolayer.

Biorelevance of Media Used During Permeability Assessment

Permeability does not only depend on drug properties and the barrier function but also on the medium present at both sides of the barrier. Therefore, the biorelevance of media used during the experiments may limit the predictive value of permeability studies (173). Traditionally, transport studies are performed in plain aqueous buffers (e.g., Hanks' balanced salt solution or Krebs-Ringer buffer, sometimes enriched with components to ensure the viability of intestinal tissue), at a fixed pH (often 7.4) in donor and acceptor compartment. Obviously, these buffers are at best only partially relevant for *in vivo* conditions. We will briefly present an overview of the available options to increase the biorelevance of media in permeability assessment (Fig. 12).

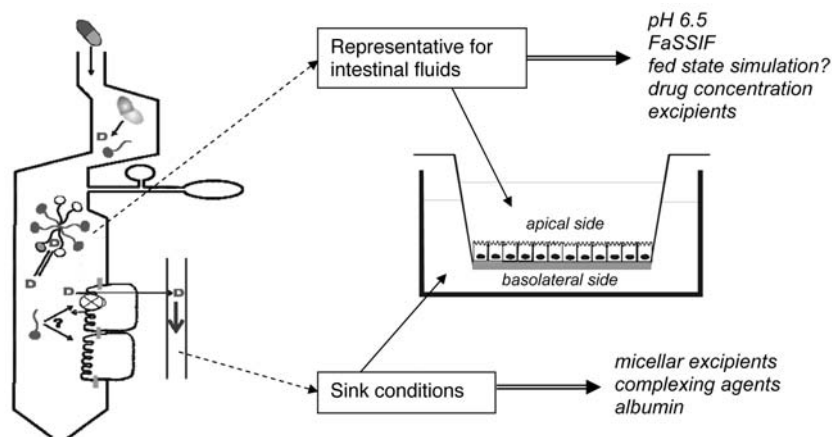


FIGURE 12 Schematic representation of the use of biorelevant media during permeability assessment.

Biorelevance of Apical Media

Complex and variable intraluminal conditions may affect both the starting conditions for transepithelial transport (e.g., increased drug concentration due to solubilization) as well as the transport process itself. Recently, several approaches have been proposed to increase the biorelevance of the media, mainly for cell culture models.

pH. The reported luminal pH of the upper small intestine under fasted conditions is generally lower than the standard apical pH during permeability assessment (7.4); this may influence the ionization and thus partitioning of drugs with a pK_a close to 7, the solubilizing capacity of micelles as well as the activity of pH-dependent transport carriers.

The use of a pH gradient in PAMPA has already been discussed in section “Description of Parallel Artificial Membrane Permeation Assay.” For the Caco-2 model system, adjustment of the apical pH to 6.5 (creating a pH gradient over the monolayer) has been proposed as a more biorelevant approach for permeability assessment (169). This pH gradient setup is recommended when performing standard screening experiments for the absorptive ranking of compounds but should be avoided when performing mechanistic bidirectional studies (65). Neuhoﬀ and coworkers demonstrated the impact of a pH gradient when performing bidirectional transport experiments of weak bases and acids in the Caco-2 system (174,175). The change of the nonionized/ionized fraction of the drugs in the presence of a pH gradient may alter the observed transport by a “false” eﬄux component for weak bases and a false influx component for weak acids.

In the diffusion chamber model, the impact of changing the pH of the mucosal buffer solution is lower than with PAMPA or Caco-2 (176). This is probably due to the presence of the mucus layer on the apical side of the intestinal tissue, which can maintain a microclimate pH regardless of the luminal pH.

Fasted-state simulation. As compared with plain aqueous buffers, intestinal fluids contain bile salts and phospholipids, creating a solubilizing environment for poorly water-soluble, lipophilic drugs. For this type of drug, the addition of bile salts and phospholipids to the apical medium in transport studies provides a physiologically relevant way to increase their donor concentration and reduce problems including detection and a low recovery (due to nonspecific adsorption). Moreover, bile salts may affect membrane fluidization or the activity of transport carriers (177).

The biorelevant dissolution medium fasted state-simulated intestinal fluid (FaSSIF, containing taurocholate 3 mM and lecithin 0.75 mM, pH 6.5, chap. 12) has been investigated as potential biorelevant transport medium in the Caco-2 system. It has been shown that FaSSIF was tolerated by Caco-2 monolayers (177,178). In a study with 19 model compounds, no effect of FaSSIF was observed on the overall predictability of the model (80). However, an impact was demonstrated on the recovery, permeability and solubility of poorly water-soluble drugs. Moreover, polarity in bidirectional transport of substrates of the efflux carrier P-gp was reduced in the presence of FaSSIF; this may be attributed to a P-gp inhibitory effect of taurocholate. A similar effect on P-gp efflux of cyclosporine and amprenavir was observed when using human intestinal fluid as the apical medium in the Caco-2 model (81,82). These observations suggest a reduced functionality of the efflux carrier P-gp *in vivo*.

The use of FaSSIF in the diffusion chamber technique is hindered by a decrease in the integrity of the intestinal tissue upon exposure to this medium (178).

Fed-state simulation. The simulation of fed-state intestinal conditions in permeability assessment is still under investigation. Patel et al. (178) developed a modified fed state-simulated intestinal fluid (FeSSIF, containing taurocholate 15 mM and lecithin 7.5 mM, enriched with glucose and glutamine, pH 6.0) that is compatible with Caco-2 monolayers but not with excised intestinal tissue. Lind et al. (179) reported the use of Leibovitz's L-15 nutritional medium, enriched with taurocholate 5 mM and lyso-phosphatidylcholine 1.25 mM as apical medium in the Caco-2 system. Furthermore, lipolytic products (oleic acid 0.5 mM and glycerol monooleate 0.25 mM) could be included in this medium without affecting the viability of the cell monolayers. The precise mechanisms by which lipids and lipolytic products alter the permeation of drugs across the intestinal barrier (e.g., by interacting with phospholipid bilayers, transporters or enzymes) may increase insight in the effect of food on drug absorption but requires further investigation (180).

Conditions after oral drug intake: drug concentrations and excipients. Intraluminal conditions after oral drug intake (e.g., presence of excipients, intraluminal drug concentrations) are the starting point for transport across the intestinal mucosa. In that respect, integration of these conditions in model systems for permeability assessment may increase the biorelevance of the system. For instance, excipients may exert a concentration-dependent effect on transepithelial transport. The presence of solubilizing excipients (e.g., complexing agents and surfactants) may result in complexation or micellar encapsulation of drugs. Although favorable for drug solubility, this reduces the free fraction of the drug in solution, which is

the driving force for diffusion across the intestinal mucosa. As a consequence, measured permeability values may decrease (181,182). In addition, surfactants may alter membrane fluidity and/or interact with carrier systems present in the intestinal monolayer. For instance, polysorbate 80, Cremophor EL and TPGS have been reported to inhibit the efflux carrier P-gp (183,76,184) resulting in an increased permeability for P-gp substrates.

The concentration of a drug in the gastrointestinal lumen is crucial as it is the driving force for transport across the intestinal mucosa. Also the contribution of saturable mechanisms, including metabolism and carrier-mediated uptake or efflux, to transepithelial transport will depend on the drug concentration. Therefore, the use of realistic drug concentrations during permeability assessment is required to investigate flux and concentration-dependent processes in a clinically relevant way. However, because of lack of knowledge about luminal drug concentrations, drug concentrations applied during permeability assessment are often based only on the compound's solubility and cytotoxicity, and on analytical considerations. This complicates the interpretation of the clinical impact of concentration-dependent processes during drug absorption.

Recently, a technique was developed to determine intraluminal drug and excipient concentrations in man after oral drug intake (185). A case study with a solubilizing formulation of the poorly water-soluble drug amprenavir clearly illustrated the importance of integrating biorelevant conditions in the *in vitro* assessment of transepithelial transport. Solubilization of amprenavir resulted in a large increase of the amprenavir flux across Caco-2 monolayers, but in a decrease of permeability. In addition, the interaction between amprenavir and the efflux carrier P-gp was reduced in the presence of intestinal fluids and completely inhibited in the presence of TPGS (82).

To integrate conditions after oral drug intake into permeability assessment, the combination of permeability models with dissolution tests may be an interesting approach. An integrated dissolution/Caco-2 system has been developed by Ginski and Polli (186). In this system, a dosage form is dissolved in a dissolution vessel; the resulting solution is transferred to a Caco-2 system and absorptive transport of the drug is monitored. Similar systems take into account the pH change in the gastrointestinal tract (187–189). Motz et al. (190) combined a flow through dissolution cell with a flow through permeation cell containing Caco-2 monolayers to evaluate complete dosage forms. In combination with biorelevant media, these systems are valuable tools in the evaluation of oral dosage forms. However, it is important to realize that they cannot completely mimic the complex gastrointestinal environment (e.g., transit and hydrodynamics).

The solvent shift method is a simple approach to simulate the pH shift in the gastrointestinal tract: the compound is dissolved in simulated gastric fluid (low pH), followed by manual transfer to the FaSSIF (pH 6.5) at the apical side of Caco-2 monolayers. This has been applied to study the absorption of the poorly soluble weak base itraconazole: after dissolution at low pH, a supersaturated solution of itraconazole was generated upon transfer to FaSSIF. This resulted in an enhanced flux of itraconazole across Caco-2 monolayers (92).

Biorelevance of Basolateral Media: Sink Conditions

In vivo, drugs absorbed across the intestinal epithelium are immediately carried away by the portal blood, maintaining the concentration gradient as the driving

force for drug transport, that is, sink conditions are preserved. In vitro, however, nonsink conditions will arise in function of transport time due to the limited volume of plain aqueous buffer in the acceptor compartment, especially for highly permeable drugs. As a result, a backward flux of drug molecules will limit transport in a nonbiorelevant way. Moreover, nonsink conditions may result in a higher cellular accumulation of drug molecules. Potential interactions with efflux carriers might be overestimated in these circumstances (191,192).

Available options to maintain sink conditions include a frequent change of the buffer in the receiver compartment or the inclusion of additives that reduce the free fraction of the drug in the acceptor compartment (e.g., albumin or surfactants).

CONCLUDING REMARKS

During drug discovery and development, gastrointestinal permeability assessment is essential to support ranking of compounds according to their "drugability," and to unravel the mechanisms underlying the absorption process. In this chapter, a variety of methods to determine permeability were discussed, including membrane-, cell- and tissue-based systems. Defining one general model for permeability assessment is not feasible. Each model has specific advantages and disadvantages, and is able to fulfill a specific need. In general, high-throughput (but less predictive) models are suitable for primary screening while low-throughput (but more predictive) models are more useful for secondary screening and mechanistic studies. More recently, the need to include more biorelevant conditions in permeability assessment has been recognized. As compared with biorelevant dissolution and solubility determination, biorelevant permeability assessment is still in its infancy. A better understanding of the intraluminal environment is paramount to defining more biorelevant media and relevant concentrations of excipients and test compounds. Rational model selection, combined with biorelevant media and thorough model validation using appropriate marker compounds, will eventually result in high-quality permeability data.

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INTRODUCTION

The Biopharmaceutics Classification System (BCS) is an approach to justify a waiver for in vivo bioequivalence (BE) of immediate-release (IR) oral solid dosage forms (1,2). According to the BCS, a drug product is characterized in terms of the solubility and permeability of the drug substance and in terms of its in vitro drug product dissolution characteristics. Solubility, permeability, and dissolution are the major determinants of the rate and extent of drug absorption from IR oral solid dosage forms. The Food and Drug Administration (FDA) and European Medicines Evaluation Agency (EMA) implemented the BCS about 10 years ago to address the regulatory question of BE. While maintaining high standards for product assessment, the BCS enables biowaivers, which allow BE assessment through the in vitro BCS guidance tests (i.e., solubility, permeability, in vitro dissolution) rather than through a human in vivo BE study. The BCS allows sponsors to request biowaivers for highly soluble and highly permeable drug substances (class I) in IR solid oral dosage forms that show rapid in vitro dissolution. Restrictions to its application include poor stability of the drug in the gastrointestinal tract, significant effects of excipients on the rate and extent of oral drug absorption, classification as a narrow therapeutic index drug, and products designed to be absorbed from the oral cavity.

One objective of this chapter is to review the implementation and impact of the BCS, including potential of future biowaiver extensions. Biowaiver extensions represent broadening of BCS class boundaries, which would effectively allow a greater number of products to be eligible for BCS-based biowaivers. Of note, neither the FDA nor the EMA has narrowed or liberalized their BCS-based biowaiver policies to date, although EMA has recently drafted revised guidelines for humans and animals that would allow for class III biowaivers (3,4).

A second objective of the chapter is to describe why in vitro studies are sometimes better than in vivo studies for assessing BE of IR solid oral dosage forms. The notion of biowaivers providing at least equal assurance of product quality as conventional human pharmacokinetic in vivo BE studies was an important element in the implementation of the BCS approach. These relative merits of BCS in vitro studies were recognized during the development of the BCS guidances, as well as during the development of companion guidances. The first regulatory reference that essentially referenced the BCS was the Scale-Up and Post-Approval Changes (SUPAC)-IR guidance, which was implemented in 1995, five years prior to the FDA BCS implementation for biowaiver (5). Fifteen years of experience with the BCS and concomitant expansion of the database available for assessing the relative merits of in vitro and in vivo BE testing enable an evidence-based assessment of the relative merits of in vitro studies compared to in vivo studies in evaluating BE of IR solid oral dosage forms.

IMPLEMENTATION AND IMPACT OF THE BCS

The first objective of this chapter is to review the implementation and impact of the BCS, including the future potential for extending the scope of the biowaiver. The following four elements are reviewed: findings from two American Association of Pharmaceutical Scientists (AAPS)/FDA workshops, activities of the World Health Organization (WHO), the International Pharmaceutical Federation (FIP) biowaiver monograph series, two EMEA draft guidelines, and the BCS in the context of the drug development and the Biopharmaceutics Drug Disposition Classification System (BDDCS).

Findings from Two AAPS/FDA Workshops

Two public workshops have been convened and cosponsored by the AAPS and the FDA since the implementation of the BCS guidance. The workshops were held in 2002 and 2007 and resulted in workshop reports (6,7).

The 2002 workshop was titled “Biopharmaceutics Classification System—Implementation Challenges and Extension Opportunities” and included a focus on four areas: methods suitability of permeability classification, solubility classification and dissolution classification, potential biowaivers for products containing BCS class II drugs, and potential biowaivers for those containing BCS class III drugs. Some findings are listed below:

1. Most notably, there was a broad consensus supporting biowaivers for at least some class III drugs whose formulations exhibit very rapid dissolution. There was consensus that biowaivers are broadly acceptable for highly soluble, very rapidly dissolving (at least 85% in 15 minutes) products.
2. There was consensus that the BCS guidance, which does not mandate any one permeability method or any prescribed set of specific experimental methods, affords flexibility and promotes the implementation of BCS across laboratories.
3. Many laboratories have not attempted to implement a permeability classification protocol that addresses all the issues recommended by the guidance. Further guidance was generally sought on approaches to demonstrate permeability method characterization and permeability system suitability. There was agreement that no single, identifiable compound has emerged as the best choice to serve as a high-permeability reference compound. While there was agreement on several permeability methods issues, more guidance is needed in identifying conditions under which permeability studies have to be performed [e.g., pH conditions, particularly when there are qualitative differences in chemistry between the drug of interest and potential high-permeability reference compound (e.g., acid vs. base)].
4. There was consensus that the minimum fraction absorbed value for high permeability can be lowered to 85%, as 90% is too conservative. There was support for an intermediate permeability class, where a drug with intermediate permeability would be one exhibiting a fraction dose absorbed between 40% and 85% and be eligible for a biowaiver.
5. Given the potential for excipient effects on intestinal motility, drug binding, or drug intestinal permeability, there was consensus that pharmacokinetic dose linearity extending sufficiently above the highest dose strength is a basis to conclude that excipients in such studies do not represent a significant risk for the drug.

6. There was consensus that the shake-flask method to assess equilibrium drug solubility is largely performed in the same fashion across laboratories. There was consensus that dissolution methodology is well established, although for quality control purposes the selection of appropriate media and sample times is often product specific and may not conform to the tests prescribed for the biowaiver procedure.
7. There was consensus that the pH range should be narrowed to include only the following pH conditions: 1.2, 4.5, and 6.8; additionally, the solubility of amphoteric compounds should be determined at the isoelectric point if it occurs between pH 1.2 and 6.8. An intermediate solubility class was suggested, given the propensity of many acids and bases to be highly soluble at pH of either 1.2 or 6.8. There was a level of consensus that a solubility volume of 250 mL is conservative, and that solubility need only be conducted between pH 4.5 and 6.8 (the pH range of the small intestine, which may be of practical benefit to some weak acids). There was no consensus about the use of solubility enhancing agents, such as surfactants, when characterizing solubility as high or low.
8. Consensus held that the rapid dissolution definition should be broadened to include products that provide no less than 85% dissolution in 60 minutes. There was consensus that the f_2 test is not necessary when the two products each provide at least 85% dissolution in 30 minutes. The f_2 acceptance criterion ($f_2 \geq 50$) can be lowered with justification that considers underlying biopharmaceutic characteristics and risk-based factors.

The 2007 workshop was titled "Bioequivalence, Biopharmaceutics Classification System, and Beyond" (7). Key highlights of the workshop were (i) a contribution describing the granting of several BCS-based biowaivers by the FDA for class I drugs whose formulations exhibit rapid dissolution, (ii) continued scientific support for biowaivers for class III compounds whose formulations exhibit very rapid dissolution, (iii) scientific support for a variety of permeability methodologies to assess BCS permeability class, (iv) application of BCS in pharmaceutical research and development, and (v) scientific progress in *in vitro* dissolution methods to predict dosage form performance.

A highlight of the workshop was the description of over a dozen BCS-based biowaivers by the FDA for class I drugs whose formulations exhibit rapid dissolution. This documentation of regulatory application of the BCS to biowaiving in 2007 contrasted with the finding from the 2002 workshop, at which time the regulatory impact of the guidance had not been substantial. In part, regulatory impact in 2002 was still low since the guidance had been issued less than two years before the 2002 workshop. Additionally, sponsors at that time were less familiar with the application and less certain of the regulatory outcome of applications based on the BCS biowaiver, and thus still preferred to use *in vivo* studies to demonstrate BE, even for drug products that could have qualified for the biowaiver.

Through 2006, the FDA BCS Committee had evaluated 25 drug products and classified 16 as BCS class I. Of the 25 drug products evaluated, 11 were new chemical entities, with 7 of these 11 receiving class I designation. Four of these 11 evaluations were at the Investigational New Drug (IND) stage. Two of the four IND drugs received class I designation and agreement on biowaivers; one received high-solubility and high-permeability designation, but dissolution was

not rapid; and for the fourth, insufficient information was provided by the sponsor. The other 7 of the 11 new chemical entities were at the New Drug Application (NDA) review stage. Five of the seven received class I designation and commensurate regulatory treatment; one was turned down; and for the seventh, insufficient information was provided by the sponsor. The remaining 14 of the 25 drug products evaluated were generics, with nine receiving class I designation. Numerous Abbreviated New Drug Applications (ANDAs) have received regulatory relief.

Examples of regulatory relief include waiver of in vivo BE studies between clinical and to-be-marketed formulations, waiver of in vivo BE study for a new strength, waiver of in vivo BE study between different strengths of to-be-marketed formulations, and waiver of in vivo BE studies for a new (solution) dosage form NDA based on the BCS knowledge of an earlier approved (tablet) NDA. The FDA BCS Committee has observed that proper integration of BCS information during drug development can save time and money.

While there have been an increasing number of successful BCS-based biowaiver applications, this progress has been attenuated by lack of international harmonization and implementation barriers within companies, including a perception of risk in project delay. The similarities and differences between the U.S. and European Union (EU) review processes were discussed at the workshop. BCS-based biowaiver criteria are generally similar between the FDA BCS guidance (1) and the EMEA note (2), but no mechanism is in place in Japan for BCS-based biowaivers (see chap. 18 for further discussion of lack of international harmonization).

The findings from these two AAPS/FDA workshops reflect progress to date in the implementation of the BCS, both in its regulatory context and as a tool in facilitating drug discovery and development. However, a significant step in BCS implementation was taken by the recent activities of the WHO.

WHO Activities and the FIP Biowaiver Monograph Series

The WHO is not a regulatory agency but directs health activities within the United Nations system. For example, WHO provides leadership on global health matters, articulates policy options, and provides technical support to countries. In 2006, WHO published an update to its advice on BE studies in its annual technical report "WHO Expert Committee on Specifications for Pharmaceutical Preparations" (8,9). A major change was the incorporation of BCS-based biowaiver, including class III biowaivers and some class II biowaivers. WHO is the only regulatory agency or major international health authority thus far that has articulated BCS-based biowaivers for either class II or class III drugs.

WHO defines high permeability as extent of absorption is at least 85%, compared to the 90% value used in the current FDA BCS guidance. WHO recognizes BCS-based biowaivers for class I drugs whose formulations exhibit rapid dissolution, class III drugs whose formulations exhibit very rapid dissolution, and class II drugs that are weak acids highly soluble at pH 6.8 and whose formulations exhibit rapid dissolution at pH 6.8 (and its dissolution profile is similar to that of the reference product at pH 1.2, 4.5, and 6.8). Compared to the current FDA BCS guidance, which is recognized to be a conservative original effort (6), the WHO BCS framework is broader, as it allows biowaivers for class III drugs, as well as biowaivers for some weak acids in class II.

A major advancement was the development of BCS data tables (9). These tables provide estimated BCS classification for the substances listed in the 14th WHO Model List of Essential Medicines (EML) of March 2005. All drugs on the EML that are administered orally are listed, along with their BCS classification, on the basis of solubility and permeability. The tables also indicate appropriate dissolution tests for biowaiver (when this procedure is applicable), potential risks, drug indications in the context of the WHO EML, and comments. This data source provides national authorities with background information on EML drugs, allowing an informed decision as to whether generic formulations should be granted a biowaiver. Many products containing drug substances on the EML are eligible for biowaiver, subject to the usage and risks in the national setting.

The FIP is the global federation of national associations of pharmacists and pharmaceutical scientists in official relations with the WHO. FIP supports the Special Interest Group on the BCS. This group has published a series of BCS drug monographs in the *Journal of Pharmaceutical Sciences*. These monographs review the literature pertaining to a specific drug, in an effort to assess whether it would qualify for application of a BCS-based biowaiver (i.e., whether a biowaiver can be recommended for a new formulation of that drug substance). Solubility, permeability, pharmacokinetics, dissolution and BE history, the therapeutic use and therapeutic window, and excipient history are reviewed.

These monographs are available from the *Journal of Pharmaceutical Sciences* (<http://www3.interscience.wiley.com/cgi-bin/jhome/68503813>), and are freely available from the FIP Web site as well (http://www.fip.org/www/index.php?page=pharmacy_sciences&pharmacy_sciences=ps_sig_bcs). To date, over 20 drugs have been subjected to this detailed BCS consideration. These include class I drugs (e.g., propranolol HCl), class II drugs (e.g., ibuprofen), and class III drugs (e.g., cimetidine HCl). Most drugs are selected from the WHO EML to help afford developing countries a means of determining whether biowaiver-based methods are suitable for assessing BE for their essential medicines.

Two EMEA Draft Guidelines

Potentially significant developments in the regulatory application of the BCS are two draft EMEA guidelines. One guideline concerns human medicines (3). This guideline is a draft update to the EMEA Note for Guidance on the Investigation of Bioavailability (BA) and Bioequivalence, which incorporates BCS-based biowaivers (2). The other guideline concerns veterinary medicines (4). This guideline is a draft update to the EMEA Guideline on the Conduct of Bioequivalence Studies for Veterinary Medicinal Products, which had not previously employed BCS-based biowaivers (10). Most notably, the EMEA Guideline on the Investigation of Bioequivalence and the EMEA Guideline on the Conduct of Bioequivalence Studies for Veterinary Medicinal Products both offer draft expansion of the BCS to include class III drugs.

The draft guidance on human medicines proposes biowaivers for IR product containing class III drugs with very rapid dissolution (>85% within 15 minutes) of the test and reference in at least pH 1.2, 4.5, and 6.8 (using 500 mL) and where excipients are qualitatively the same and quantitatively very similar between test and reference. To an extent, this BCS extension follows from previous workshops and WHO guideline. The use of 500 mL of dissolution media

rather than 900 mL, and the requirement that excipients be qualitatively *and* quantitatively similar are significant limitations to application of the biowaiver.

The draft guidance on human medicines proposes biowaivers for IR product containing class I drug (high solubility; extent of absorption $\geq 85\%$) with very rapid dissolution ($>85\%$ within 15 minutes) of the test and reference in at least pH 1.2, 4.5, and 6.8 (using 500 mL), and where excipients are not suspected of having any relevant impact on bioavailability (BA). An 85% lower limit for high permeability is proposed. However, other aspects (e.g., the requirement that dissolution be very rapid in 500 mL vs. rapid dissolution in 900 mL) are significant limitations and not consistent with the current EMEA note (2) or FDA guidance (1).

BCS in Drug Development and the Biopharmaceutics Drug Disposition Classification System

BCS has provided a significant impact in drug discovery and development, where there has been a growing recognition to design “drug-like” properties into new chemical entity programs. However, disease targets are increasingly hidden behind hydrophobic “barriers,” such that drug design must be increasingly sophisticated to simultaneously ensure potency and overcome barrier issues. As the target dose in man is always a source of great uncertainty during early development, application of the BCS will always be less precise in early development.

Nevertheless, drug biopharmaceutics properties are being integrated into quantitative and predictive models of dosage form pharmacokinetic performance, guiding the selection of drug candidates, active pharmaceutical ingredient (API) processing and form selection, and dosage form technology. For example, Ku describes the use of the BCS in early drug development (11), where biopharmaceutic characteristics are used for preliminary BCS classification of pipeline compounds. A decision strategy is described to facilitate early development, including a BCS-based animal formulation development decision tree. Compounds are allocated into one of five formulation strategies, with the goal of consistent pharmacokinetic performance and avoiding bridging BA/BE studies.

Cook et al. describe several examples where application of the BCS has been beneficial, including obtaining biowaivers as well as facilitating formulation development during the clinical development cycle (12). In a case study of pregabalin, BE needed to be studied near the time of submission. Three different formulation series comprised 11 different strengths. A strategy was devised to compare dissolution profiles of the highest and lowest strengths of each series. An in-house educational effort, along with interactions with FDA scientists, allayed in-house concerns about the less familiar BCS as compared to the traditional *in vivo* BE approach. The subsequent BCS class I biowaiver resulted in filing over one month earlier, with a savings of more than \$1 million compared with a more traditional approach that would have utilized four separate BE studies.

Yamashita and Tachiki showed that BCS classification can be useful in promoting an efficient and cost-saving strategy for oral drug product development (13). The risk factors that cause bioinequivalence in BE studies were analyzed by considering BCS classification for 44 generic products. It was found that for classes I and III drugs, risk of bioinequivalence risk could be predicted

from the ratio of AUC/dose, a parameter which can be readily estimated for postapproval changes and proposed generic drugs.

With such examples it seems likely that the BCS will become an invaluable tool in the future, especially given the continued industry emphasis on more efficient discovery and decreasing drug development timelines. BCS is also being applied to aid the consideration of a drug's biopharmaceutical properties in the context of the review paradigms of Quality by Design (QbD) and Question-based Review (QbR).

Another recent development is the proposal of the BDDCS as a means to predict permeability classification (14). According to this paradigm, when metabolism is the major route of drug elimination, the drug exhibits high permeability. By contrast, if renal and biliary excretion of unchanged drug is the major route of elimination, the drug should be classified as low permeability. The BDDCS was proposed to predict the in vivo disposition for all four classes, as well as increasing the number of class I drugs eligible for BE study waivers.

Since the proposal of a BDDCS, it has been further recommended that extent of drug metabolism (i.e., $\geq 90\%$ metabolized) serve as an alternate method in defining class I drugs substances, where $\geq 90\%$ metabolized is an additional methodology that may be substituted for $\geq 90\%$ absorbed (15). Metabolism $\geq 90\%$ can be concluded when mass balance of the phase 1 oxidative and phase 2 conjugative drug metabolites in the urine and feces account for $\geq 90\%$ of dose, after a single oral dose to humans at the highest dose strength. Chen and Yu analyze preclinical and clinical data of 51 BCS high-permeability drugs, examining drug metabolism as a tool for supporting and extending current BCS classification (16). All 51 compounds were of high permeability. While a majority showed high metabolism, 14 of 51 drugs had poor metabolism, indicating that high permeability, as defined by BCS, does not necessarily dictate extensive metabolism. The drugs with high permeability but poor metabolism were broadly low molecular weight hydrophilic compounds and were likely to be absorbed by active transport mechanisms. However, the extent of drug metabolism appears useful in supporting permeability classification under some situations.

To summarize the discussion related to the implementation and impact of the BCS, findings from two AAPS/FDA workshops, WHO activities and FIP biowaiver monograph series, two EMEA draft guidelines, and BCS in drug development and the BDDCS suggest an increasingly important role for the BCS and possible future changes in its scope of application.

ADVANTAGES OF IN VITRO BE TESTING OVER IN VIVO BE TESTING

The second objective of this chapter is to discuss situations in which in vitro studies are better than in vivo studies for assessing the BE of IR solid oral dosage forms (17). In vitro studies can be advantageous in terms of (i) reducing costs, (ii) more directly assessing product performance, and (iii) offering benefits in terms of ethical considerations. Situations favoring in vitro testing include class I drugs in products with rapid dissolution (i.e., 85% in 30 minutes or less in pH 1.2, 4.5, and 6.8 media), class III drugs in products with very rapid dissolution (i.e., 85% in 15 minutes or less in pH 1.2, 4.5, and 6.8 media), and highly variable drugs (HVDs) in products that are rapidly dissolving and are historically unproblematic in terms of BE.

In Vitro Studies Reduce Costs

In vitro studies reduce costs through avoiding in vivo studies where BE is self-evident, where biopharmaceutical data anticipates BE, and where in vivo BE study type II error is high. The need to reduce the cost of drugs is motivated by research that indicates that up to 32% of elderly adults take fewer drugs than prescribed due to cost (18–20). Not surprisingly, this cost-related medication noncompliance prevents some patients from achieving the full therapeutic benefits of therapy and can result in more use of emergency and institutional services (19,20).

Rapidly dissolving IR formulations of solid dosage forms of class I drugs represent scenarios where BE is self-evident. Cook and Bockbrader examine the potential cost savings of using BCS-based biowaivers for class I drugs in lieu of in vivo BE testing (21). They assumed that 25% of BE studies are for class I drugs. They conservatively estimated in 2002 that “there is the potential to save one quarter of the annual expenditures on bioequivalence studies, \$22 to \$38 million dollars/yr” in direct costs of testing. Additional indirect savings can occur if BE studies are rate limiting to drug regulatory submission (e.g., avoid lost sales of over \$1 million/day if product leads to sales of \$400 million/yr) and if opportunity costs are considered (e.g., resources not deployed to running in vivo studies can be redeployed to other projects).

Since the primary regulatory concern about BE is to protect patients against the possibility that products that are not BE might be approved by mistake (22), an appropriate question is how often products containing class I drugs have passed the tests for rapid dissolution but failed in vivo BE testing. Figure 1 illustrates type I and type II errors in the context of BE testing. Assuming conventional in vivo BE testing using human pharmacokinetics is a perfect indication of whether products are BE; the chance that a product containing a class I drug will exhibit rapid dissolution but fail in vivo BE testing constitutes a type I error. Two presentations from the FDA at workshops have reported no documented BE failures for class I drugs in the United States (23,24). A scientist at RIVM in the Netherlands has also indicated that there are no known BE failures for class I drugs in the European Union [Dirk M. Barends (Rijksinstituut voor Volksgezondheid en Milieu, Netherlands), personal communication, March 2007]. It thus appears that the risk of type I errors through the use of in vitro testing to assess BE of class I drugs in the United States or European Union is extremely low.

H_0 : Products are not bioequivalent.

H_1 : Products are bioequivalent.

		Truth	
		H_0 is true	H_0 is false
Result of Testing	Fail to reject H_0	Good	Type II (producer risk, β)
	Reject H_0	Type I (consumer risk, α)	Good

FIGURE 1 BE, hypothesis testing, and errors. In BE testing, the null hypothesis states that products are not BE, while the alternate hypothesis states that products are BE. Type I error occurs when products are erroneously concluded to be BE when they are not BE. Type I error represents a risk to the consumer (i.e., a health risk to the patient). Type II error occurs when products are erroneously concluded to be not BE when they are BE. Type II error represents a risk to the producer. *Abbreviation:* BE, bioequivalence.

While the risk of type I error from *in vitro* testing is an important consideration, *in vitro* dissolution testing is frequently overly discriminating in that failed *in vitro* testing does not indicate lack of *in vivo* BE. For example, metoprolol tartrate formulations were BE *in vivo*, although comparison of their dissolution profiles yielded f_2 values less than 50 and the slow formulation failed the USP dissolution specification (25). Formulation studies of the class III drugs ranitidine (26) and cimetidine (27) also showed BE among formulations where *in vitro* dissolution detected differences. Dissolution studies of various marketed doxycycline hyclate formulations also exhibited sensitivities to formulation, even though products demonstrated BE *in vivo* (28). Such reports indicate that *in vitro* testing of many IR products are overdiscriminating rather than underdiscriminating in terms of BE.

In addition to scenarios where BE is self-evident, *in vitro* studies achieve reduced costs through avoiding *in vivo* studies where biopharmaceutic data anticipates BE, such as studies of rapidly dissolving IR formulations containing a BCS class III drug. As discussed above, scientific consensus supports biowaivers for at least some class III drugs whose formulations exhibit very rapid dissolution. However, potential concerns about class III biowaiver merit addressing (7). A comprehensive analysis of results of conventional human pharmacokinetic *in vivo* BE testing of class III IR products, similar to what has previously been presented (23), would be beneficial. Such analysis has potential to measure the type I error of *in vitro* BE testing for BCS class III drugs.

Direct cost savings from BCS-based biowaivers for class I drugs have been conservatively calculated to be \$22 to \$38 million/yr, assuming 25% of BE studies are for class I drugs (21). Applying the same analysis to class III drugs and assuming 25% of BE studies are for class III drugs (14,29,30), another \$22 to \$38 million/yr could be directly saved by employing BCS-based biowaivers. Together, biowaivers for class I and III drugs have the potential to directly save \$44 to \$76 million/yr in *in vivo* BE study expenditures. The assumption that 50% of established drugs are either class I or III is reasonable, if not conservative. Takagi et al. provisionally BCS classified the orally administered IR drug products in the top 200 drug product lists from the United States, Great Britain, Spain, and Japan. From these four lists, compounds were 30% to 36%, 30% to 34%, 19% to 28%, and 3% to 7% in BCS class I, II, III, and IV, respectively (29). More than 50% on each list were determined to be high-solubility drugs (55–59%). This observation agrees with that of Benet and Wu, who extensively examined 169 drugs in the WHO EML. These 169 compounds showed 39%, 30%, 26%, and 8% for BDDCS class I, II, III, and IV, respectively (14). These distributions are further supported by Khandelwal et al., where drug disposition data for 56 previously unclassified drugs was obtained from an extensive literature search (30). These 56 compounds were distributed within BDDCS class I, II, III, and IV as 47%, 20%, 25%, and 9%, respectively.

In vitro studies can also reduce costs through avoiding *in vivo* studies where *in vivo* BE study type II error is high. HVDs are drugs with high within-subject variabilities ($\text{ANOVA-CV} \geq 30\%$) in $C_{\max}$ and/or AUC (31). HVDs typically have flat dose response curves and large therapeutic windows, such that clinically important adverse drug reactions (ADRs) occur at much higher doses than those required for efficacy. Currently in the United States, the same conventional BE statistical analysis is applied to HVDs, as well as non-HVDs. In *in vivo* BE studies with HVDs often require a much greater number of subjects

than non-HVDs, to avoid type II error. Figure 1 illustrates type II errors in the context of BE testing. Type II error occurs when products cannot statistically be shown to be bioequivalent even though they are. High variability is a frequent basis for low in vivo BE study power, necessitating larger number of subject to achieve sufficient power. Tanguay et al. examined over 1200 BE studies performed between 1992 and 2002 (32) and observed that "drug formulations associated with an intraindividual variability of 35% or more failed to meet BE criteria at an astronomic rate of 85%."

In spite of this pattern of high in vivo BE testing failure for HVDs, high variability is frequently not due to poor product quality, although the identification of products with poor quality is a central goal in BE testing. Davit et al. collected data from all in vivo BE studies reviewed at FDA's Office of Generic Drugs from 2003 to 2005 (33). The review entailed over 1000 in vivo BE studies of 180 different drugs, of which 31% were highly variable. Of these HVDs, 51%, 10%, and 39% were either consistently, borderline, or inconsistently highly variable, respectively. Drug substance pharmacokinetic characteristics and drug product dissolution were considered to cause high variability. About 60% of the HVDs were highly variable due to drug substance pharmacokinetic characteristics. Formulation performance contributed to the high variability only about 20% of the time.

This perspective that conventional human pharmacokinetic in vivo BE testing is problematic, costly, and inconclusive for HVDs has motivated the development of several novel in vivo BE methodologies and possible alternative acceptance criteria for HVDs. Buice et al. (34) state "Unreasonable BE costs, necessitating excess studies can only increase this [consumer] cost. . . . Findings further suggest that the 90% confidence interval criteria should be adjusted for highly variable drugs." Rather than relaxing the BE criteria, it is suggested here that in vitro BE testing may be a better approach for HVDs, particularly if the drug's biopharmaceutic properties are favorable and formulation performance is not suspected.

Estimating the potential direct cost savings by employing BCS-based bio-waivers for HVDs is complicated by several factors. One factor is that in vivo BE testing of HVDs uses larger number of subjects than that for testing of non-HVDs. Another factor is that in vivo BE studies with increasingly larger numbers of subjects (i.e., drugs with increasing larger variability) suffer from the highest rates of failure, largely due to type II error. For example, the failure rate of studies using $n = 49$ to 60 subjects was three times larger than the failure rate of studies using $n = 37$ to 48 subjects (32). These data imply that approaches to increased sample size to accommodate HVDs are far from efficiently practiced, rather than a refutation of classical statistics that anticipates reduced type II error with larger number of subjects. Because of the high subject numbers per study and the higher failure rate of studies with HVDs, the potential direct cost savings for HVDs could be much larger than that for either class I or class III drugs, which suffer less from this encumbrance, since most class I and class III drugs are not HVDs. Potential indirect savings (e.g., more rapid product development by reducing erroneous BE failures) also seems both likely and desirable.

In summary, in vitro studies can sometimes serve as a better method than conventional human pharmacokinetic in vivo studies due to reduced costs by avoiding in vivo studies where BE is self-evident, where biopharmaceutic data anticipates BE, and where in vivo BE study type II error is high.

In Vitro Studies More Directly Assess Drug Product Performance

A second reason for preferring in vitro over in vivo BE studies is that in vitro studies more directly assess product performance than do conventional in vivo BE studies. In vitro studies focus on comparative drug absorption from the two products, while in vivo BE testing can suffer from complications due to its indirect approach.

Drug absorption is composed of the processes of drug release from the dosage form (i.e., dissolution) and drug permeation through the gastrointestinal mucosa. While the pharmacokinetic metrics $C_{\max}$ and AUC are by far the most common measures to assess BE in practice, neither the definition of BE nor the definition of the BE requirement (35) references $C_{\max}$ or AUC, or even refers to pharmacokinetic plasma profiles. Neither definition necessarily requires in vivo studies. Rather, $C_{\max}$ and AUC are commonly used as metrics for the rate and extent of drug absorption. The definitions of BA (35) and bioequivalent drug products (22), as well as the conditions under which products are considered bioequivalent (36), feature drug absorption rather than pharmacokinetic plasma profiles.

In vitro studies more directly assess drug absorption than do in vivo BE studies. In vitro dissolution methods and in vitro (and in situ) permeation methods are now well established. Compendial dissolution equipment is standardized. In vitro (and in situ) permeation methods are used in many laboratories throughout the world at various stages of drug development, from screening in early discovery with respect to permeability to regulatory applications in seeking BCS-based biowaivers for products containing class I drugs (2).

Limitations exist in in vitro dissolution testing and in vitro (and in situ) permeability testing. For example, there is no single universal dissolution medium that a priori predicts in vivo drug dissolution. There is no single in vitro (or in situ) permeability test condition that mimics the complex intestinal mucosa that the drug can “see” over the course of its passage through the gastrointestinal lumen. Nevertheless, multicondition dissolution testing and multicondition permeability testing address such limitations by using a number of test conditions (e.g., multiple pH levels). Multicondition in vitro dissolution and permeation testing within a drug absorption conceptual framework provides a focus on comparative drug absorption, where in vitro results have in vivo meaning in comparing products, including direct relevance to the term bioequivalent drug products and conditions under which products are considered bioequivalent.

Conventional human pharmacokinetic in vivo BE testing suffers from complications due to its indirect approach. Pharmacokinetic plasma profiles represent an indirect approach to measure drug absorption. Postabsorption events such as metabolism and enterohepatic recycling can result in complex and variable pharmacokinetic profiles, and can have little relevance to drug product quality or the rate and extent of drug absorption. About 60% of HVDs are highly variable due to drug substance pharmacokinetic characteristics, rather than drug product characteristics (33). In particular, in this comprehensive survey, it was reported that 83% of drugs that exhibit consistent or borderline high variability showed extensive first-pass metabolism. Meanwhile, only 21% of drugs that are not highly variable show extensive first-pass

metabolism. This survey, in concert with the high rate of type II error for HVDs (32), indicates that extensive first-pass metabolism can be a confounding factor in comparing drug absorption between products and that pharmacokinetic-based evaluations of such products may therefore be a poor approach to comparing product quality. Within context of BE, it should be noted that, while under some circumstances the extent of first-pass metabolism can depend on dissolution rate when the first-pass metabolism is saturable in the usual dose range, there are few documented cases of this in the literature (e.g., propranolol sustained release vs. IR products).

Enterohepatic recirculation is also a postabsorption process that can modulate plasma profiles. It can cause drug to be secreted into bile after primary drug absorption, where drug is then exposed to the gut again, from which drug can be reabsorbed. This secondary absorption can result in a second peak in the plasma profile and further plasma drug exposure. For drugs that are enterohepatically recycled, the hepatobiliary system impacts plasma profile kinetics, introducing a nonproduct performance-related factor into the evaluation. Within the context of BE, there appears to be no evidence that the enterohepatic recycling of drugs is formulation dependent.

An additional scenario where *in vivo* BE testing suffers from its indirect approach is when the *in vivo* BE testing employs multiple dosing (e.g., drug toxicity is high, such that only patients on maintenance therapy are allowed to participate). Pharmacokinetic profiles from multiple dosing typically reflect not only the most recent dose but also several of the most recent doses. As a result, multiple-dosing *in vivo* BE studies are viewed as less sensitive than single-dose *in vivo* BE studies.

These complications of conventional human pharmacokinetic *in vivo* BE testing manifest in the lack of a single standard for *in vivo* BE. The numerous BE criteria and proposals reflect the fact that *in vivo* BE testing is not a direct assessment of product performance, but rather an indirect assessment that can be confounded by nonproduct factors [e.g., within-subject variability in absorption, distribution, metabolism, and excretion (ADME)]. For example, the Canadian agency does not require a confidence interval for C_{max} , but corrects for drug content; FDA requirements differ on this point. The CPMP/EMA guideline allows broadening the BE limits (e.g., 75–133%) under certain situations. There are also proposals to broaden the BE limits according to the within-subject variability of the reference. Additionally, *in vivo* BE testing is subject to metric issues, with C_{max} not being viewed as an ideal metric for rate of absorption. As a result, there is sometimes a need to measure early exposure. These limitations of *in vivo* BE testing have been repeatedly and frequently discussed, resulting in a range of different criteria to assess BE from pharmacokinetic data.

In summary, a second reason that *in vitro* studies are sometimes the better BE method is that they often more directly assess product performance than do conventional human pharmacokinetic *in vivo* BE studies. *In vitro* studies can more directly focus on the step that addresses drug absorption from the two products than does a comparison of pharmacokinetic profiles, especially if multicondition *in vitro* dissolution and permeation testing are implemented. *In vivo* BE must be viewed especially critically for drugs with high first pass, HVDs, those with enterohepatic cycling, and where multiple-dose studies are used to assess BE.

In Vitro Studies Offer Benefits in Terms of Ethical Considerations

A third reason is that in vivo studies better embrace the principle “No unnecessary human testing should be performed” and can result in faster development. In vivo BE testing is generally safe, in cases where the majority of ADRs are mild (37). BE studies after the drug has been approved as safe and effective can be expected to be generally safe. Adding to this safety is that conventional in vivo BE testing is single dose, limiting drug exposure. However, ADRs have occurred in BE testing, for example, for aripiprazole, which is used to treat schizophrenia and bipolar I disorder. The reference listed drug (RLD) for aripiprazole is now the 5-mg tablet and not the 30-mg strength (22). The 30-mg strength caused ADRs in healthy volunteers, such that the lowest strength rather than highest strength is now used in BE testing of aripiprazole [Chris Hendy (Novum Pharmaceutical Research Services, Pittsburg, PA), personal communication, March 2007]. Serious ADRs have also occurred in BE testing of clozapine. The FDA guidance on clozapine BE testing (38) reads

In the 1996 guidance, the Agency recommended that doses of clozapine tablets be administered to healthy subjects ... Because a high number of healthy subjects experienced serious adverse effects such as hypotension, bradycardia, syncope, and asystole during clozapine bioequivalence studies, FDA is recommending that studies not be conducted using healthy subjects. In addition, a single-dose study using a 12.5 mg dose is no longer recommended. Instead, this guidance recommends a multiple-dose bioequivalence study conducted in patients using the highest dosage strengths (e.g., 100 mg tablets).

BE testing frequently occurs during product development, prior to NDA filing. A typical NDA includes three to four BE studies (21,39). A persistent question is “what risk level is acceptable in research studies performed in healthy volunteers” (40). Peroxisome proliferator-activated receptor (PPAR) agonists are a drug class with significant potential. Over 50 INDs of PPAR agonists have commenced. However, numerous development programs of PPAR agonist have been terminated due to safety concerns (41). In 1997, troglitazone was approved and then removed three years later because of liver failure. While it is not evident that BE studies of experimental compounds have caused major ADRs, the philosophy that no unnecessary human testing should be performed would seem to favor in vitro BE testing over in vivo BE testing when in vitro BE testing is suitable, particularly if compound safety has not been established.

Is it ethical to conduct an in vivo BE test for an IR solid oral dosage form containing a BCS class I drug that would otherwise receive a BCS-based bio-waiver? It would appear difficult to argue that the answer is “yes.” Is it ethically desirable to replace in vivo BE testing with in vitro BE testing? Conclusions drawn in the area of animal testing may provide some insight into this basic question. Institutional Animal Care and Use Committees (IACUCs) strongly promote the replacement of animal testing with non-animal alternatives. In proposing animal testing to IACUC, investigators typically must describe potential alternatives to animal testing, including why such alternatives are not preferred. Investigators must also show that the proposed animal testing does not cause unnecessary duplication. Investigators must typically cite literature searches using two different databases that indicate poor suitability of in vitro

and/or computer modeling alternatives. A corollary to the question “Is it ethically desirable to replace in vivo BE testing with in vitro BE testing?” is “Should Institutional Review Boards (IRBs) strongly promote the replacement of in vivo BE testing with non-in vivo BE testing alternatives?” It would seem that the answer is “yes.”

Preapproval BE studies are common within a development program. A typical NDA includes three to four BE studies (21,39) and can be rate limiting to drug development. One situation is when BE study results are needed before any further product development (21). Another situation is the final BE study, which is the last document needed for NDA filing (42). In vitro studies can be typically completed in less time (e.g., two months) than an in vivo BE study. In addition to having financial implications for the sponsor, these delays have implications for patients and the impact of ethical considerations of making therapies available to patients as soon as possible.

In summary, in vitro studies often offer benefits in terms of ethical considerations. In vitro studies better embrace the principle “No unnecessary human testing should be performed” and can result in faster development.

Situations When In Vitro BE Testing Is Preferred

Situations when in vitro BE testing should be viewed as preferred over conventional human in vivo BE testing include class I drugs with rapid dissolution, class III drugs with very rapid dissolution, HVDs with rapid dissolution, and drugs that have hitherto not shown BE problems.

The scientific basis for BCS-based biowaivers of IR solid oral dosage forms containing a class I drug is well accepted (1,2,6–9). Such biowaivers require test product to exhibit rapid dissolution (i.e., 85% in 30 minutes or less) in pH 1.2, 4.5, and 6.8 media, to dissolve similarly to reference, and to contain only certain types and quantities of excipients, along with other requirements (e.g., therapeutic index). Scientific support continues for biowaivers for class III compounds whose formulations exhibit very rapid dissolution (i.e., at least 85% in 15 minutes). Rationale for such class III biowaivers is that these products with very rapid dissolution perform like an oral solution in vivo, since intestinal permeability limits drug absorption. This rationale is further supported by the regulatory practice of allowing biowaivers of oral solutions of class III drugs (43).

In vitro BE testing is preferred over in vivo BE testing for HVDs with rapid dissolution and that are not bio(equivalence)problem drugs. As mentioned earlier in this chapter, over 30% of drugs are highly variable (33). HVDs with rapid dissolution and that are not bio(equivalence)problem drugs appear to be excellent candidates for in vitro BE testing, since they typically have flat dose-response curves and large therapeutic windows and therefore generally low safety concerns. Such products would benefit from in vitro testing since in vitro testing reduces costs, more directly assesses product performance, and offers benefits in terms of ethical considerations.

It should be noted that in vivo BE is not even recommended in all cases in current practice. Pharmacokinetic BE studies are waived in many cases for lower doses, per 21 CFR 320.22(d)(2) based on (i) acceptable BE studies on the highest strength, (ii) proportional similarity of the formulations across all strengths, and (iii) acceptable in vitro dissolution testing of all strengths (35). Additionally, the FDA allows SUPAC changes in excipients, manufacturing site, manufacturing

batch size, and manufacturing process/equipment to be allowed based on in vitro tests, for both IR and modified release products (5,44,45).

In vitro BE testing has a long history of use. 21 CFR 320.33 has provided criteria to assess actual or potential BE problems. In the latter 1970s, drug products that had met these criteria were deemed "bioproblem" drug products. In vitro studies were expected to correctly assess BE for products that were not bioproblem drug products. For IR products not containing a bioproblem drug, FDA allowed drug efficacy study implementation (DESI)-effective drugs to be assessed for BE through in vitro studies alone. DESI was a program initiated in the 1960s to classify all pre-1962 drugs as either effective, ineffective, or needing further study. Since 1979, such products that passed BE testing were assigned an AA rating in FDA's "Approved Drug Products with Therapeutic Equivalence Ratings." 21 CFR 320.24 also describes situations when in vitro studies can be used alone to document BE.

Like the United States, Germany has had a history of using in vitro testing as a surrogate for in vivo BE testing. The German drug agency BfArM described situations when in vivo BE studies are not needed (46). A decision tree was based on pharmacodynamic, pharmacokinetic, and physicochemical criteria. In describing the use of this approach in Germany (46), Gleiter et al. indicate the names of 90 drugs for which in vivo BE studies were not generally required, as well as the names of 120 drugs for which in vivo BE studies would be requested. However, the decision tree allowing biowaivers for oral IR and solution dosage forms was withdrawn in 2003 after over 15 years of use, to facilitate European Union harmonization [Dirk M. Barends (RijksInstituut voor Volksgezondheid en Milieu, Netherlands), personal communication, March 2007].

Future Considerations

Always requiring or preferring in vivo demonstration of BE over in vitro methods is not rational and not scientific. For a rapidly dissolving IR solid oral dosage form containing a class I drug, it would be difficult to justify why in vitro BE test is not preferable over the conventional human pharmacokinetic in vivo BE testing.

Situations when in vitro testing should be viewed as preferred include class I drugs with rapid dissolution, class III drugs with very rapid dissolution, and HVDs with rapid dissolution and that are not bio(equivalence)problem drugs. These situations represent a substantial majority of drugs. Class I and III drugs make up about 50% of all marketed oral solid dosage forms (14,25) and upwards of 30% of drugs are HVDs (29). Since most HVDs show high first-pass metabolism (29) and since many such drugs may be expected to be highly permeable (14), it can be estimated that a substantial majority of drugs are candidates for in vitro BE testing as the better BE test. Sponsors of potential in vivo human pharmacokinetic BE testing should be required to justify why in vitro data is insufficient, similar to proposals for animal testing, which require justification for not employing an in vitro approach.

Any effort to more broadly employ an in vitro approach would benefit from publicly available analysis of the relative performances of in vitro BE testing and in vivo BE testing. There remain uncertainties among pharmaceutical companies and regulatory authorities on how to demonstrate the requirements for BCS-based biowaivers.

Type I errors of in vitro testing would be an obvious concern. Analyses, such as those previously performed and described (23), should be continuously

updated and disclosed. In particular, written analyses would be most helpful, with due consideration to the fact that generic drug companies do not currently need to submit failed BE studies to the FDA. Ongoing open discussions about best practices in permeability classification (7,15) should be continuously encouraged. A better biopharmaceutical understanding of dosage form performance and kinetic role of in vivo dissolution in overall oral drug absorption is needed (47). More examples of detailed descriptions of how dosage forms achieve drug release in vivo are welcome. Better understanding of when and how in vitro dissolution methodologies do and do not reflect in vivo dissolution is needed. While type I errors of in vitro testing is an obvious concern, a database for type II errors from in vitro dissolution would also be valuable. Ideally, QbD efforts during product development will help address some of these needs. Other topics needing better understanding are type II errors in current in vivo BE testing, which could be a major source of discordance between in vitro and in vivo BE results.

The path forward also requires a global effort, since many major products are registered worldwide. If one agency allows in vitro testing and another requires in vivo testing, in vivo testing will always be performed, even if in vitro testing is the better test. This lack of harmonized acceptance criteria is an obstacle that hinders wider utilization of in vitro testing (48).

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Dissolution Testing to Forecast In Vivo Performance of Immediate-Release Formulations

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INTRODUCTION

To facilitate development and to ensure the quality of drug products, it is desirable to have in vitro test systems that can be used to forecast their in vivo behavior. Of the quality control tests generally described in pharmacopeias, dissolution tests seem to be the most closely associated with in vivo performance of oral drug products. This is principally because the release/dissolution step is prerequisite to the drug absorption process from many dosage forms administered orally.

Many monographs for solid oral dosage forms in the U.S. pharmacopeia (USP) contain a section on dissolution testing as a part of the routine quality control tests for the product in question. The conditions of the test described therein are often based on tests proposed by the innovator and are subsequently used for the abbreviated new drug applications of generic pharmaceutical products. In most cases the proposed dissolution media are simple aqueous buffers and the dissolution apparatus is either the USP apparatus 1 (basket method) or the USP apparatus 2 (paddle assembly) (1). These quality control dissolution test conditions often deviate considerably from the gastrointestinal (GI) tract physiology, and hence the results often do not translate directly into the in vivo performance of the dosage form.

In general, to be able to predict what happens intraluminally after oral administration of the dosage forms, for example, to develop an in vitro-in vivo correlation (IVIVC), the dissolution test conditions have to be carefully designed to adequately resemble the physiological environment of the GI tract. The main question that arises is "How closely could and should we approach the physiological conditions?" Applying the Biopharmaceutics Classification System (BCS) (2), the rate-determining step to drug absorption can be defined and, based on this step, the likelihood of developing a meaningful IVIVC can be assessed. For immediate-release (IR) drug products containing highly soluble compounds, that is, those belonging to class I or class III of the BCS, there is little sensitivity to the dissolution test conditions and the use of simple aqueous buffers and appropriate test parameters, for example, as suggested by biowaiver guideline (3,4), are often sufficient to assess bioequivalence (BE) of drug products. By contrast, products containing BCS class II or class IV compounds are likely to be more sensitive to the dissolution test conditions. Therefore, the use of biorelevant dissolution media and appropriate apparatus representing the GI hydrodynamics appear to be more appropriate, and it may be possible to

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establish an IVIVC. To evaluate dissolution of these poorly soluble compounds, several factors should be taken into consideration. For those drugs that are ionizable in the pH range of the GI tract, the pH of the dissolution media may be of great importance. In addition, naturally occurring surfactants, that is, bile secretions, food components, and other relevant components as well as motility patterns and hydrodynamics can affect dissolution of poorly soluble drugs considerably.

Two crucial issues that should be taken into account for establishing the *in vitro* biorelevant dissolution test conditions include (i) the “composition of biorelevant dissolution media,” to reflect the composition of the gut lumen contents at the site of dissolution and (ii) the “hydrodynamics” of the tests, to reflect the motility in the GI tract. An overview of these two parameters with regard to the ability to predict the performance of an IR dosage form *in vivo* is given later in the text and the details are further discussed in this chapter.

In 1998, Dressman et al. (5) published a comprehensive discussion of the physiological aspects that are important to establish and apply biorelevant dissolution tests, and Galia et al. (6) applied this concept to the prediction of *in vivo* performance of IR pharmaceutical products. Perhaps the most important development at that time was the introduction of two media representing the fluids in the proximal part of the small intestine in the pre- and postprandial states, namely (i) fasted-state simulated intestinal fluid (FaSSIF) and (ii) fed state-simulated intestinal fluid (FeSSIF). These media have been widely used since then, both in academic and industrial spheres. Since that era, various improvements on the composition of media simulating the upper small GI lumen have been proposed (7–9).

Interestingly, simulation of luminal hydrodynamics was an issue much earlier than that of luminal composition, when it was shown that disintegration rather than dissolution could be more important for some dosage forms (10). However, since then only limited attention has been devoted to the hydrodynamics and mechanical conditions simulating the luminal conditions. Lack of a precise knowledge of the luminal hydrodynamics and their complexity are two major reasons for this long lull in progress. During the last decade, however, our knowledge of luminal motility, volumes, and flow rates has been improved substantially and, as a result, various proposals for modeling luminal hydrodynamics that deserve further evaluation have been made.

This chapter is divided into two main sections: the first deals with the biorelevant dissolution media and the second with the biorelevant hydrodynamics of the dissolution test. Since the focus in this chapter is IR formulations, both sections deal with biorelevant conditions in upper GI lumen (stomach and proximal small intestine) only. Relevant considerations for the lower gut are provided in chapters 9 and 13.

BIORELEVANT DISSOLUTION MEDIA

Dissolution Media to Forecast Dosage Form Performance in the Stomach

Although the stomach is not a quantitative site of absorption for most drugs, it is the main region where IR drug products disintegrate (e.g., IR tablets, IR capsules) and/or disperse (lipid-based formulations) after oral administration. It thus serves as a reservoir from which the disintegrated/dispersed drugs enter the small intestine.

Upon meal ingestion, the intragastric environment changes considerably. The different conditions between the fasted and fed stomach can lead to differences in solubility and dissolution of drugs and drug products pre- and postprandially. Hence, to adequately predict the *in vivo* intragastric performance of the dosage forms, the *in vitro* test parameters should correspond to these conditions appropriately.

Fasted State

It is well known that under fasting conditions the healthy human stomach usually has an acidic pH, ranging between one and three (11,12). This is due to a basal secretion of gastric acid. In other cases, for instance, in a certain percentage of elderly, in patients receiving antacids or gastric acid blockers, and in achlorhydric patients, the fasting gastric pH value is elevated. A physiologically acidic environment in the stomach can be of importance for the dissolution of poorly soluble weakly basic compounds but is not so relevant to the poorly soluble weakly acidic compounds, since in the fasted state the weakly basic drugs dissolve primarily in the stomach while the weak acids will remain largely undissolved. *In vitro*, simulated gastric fluid (SGF) described in the pharmacopeias (13–15), with a pH of 1.2 and containing pepsin (3.2 mg/mL), has been used as a dissolution medium to simulate the human fasting stomach for many years. It serves well as a test medium, for quality control purposes, for many drug products. However, upon comparison with the *in vivo* parameters, this simple aqueous media may not be appropriate for estimating the drug dissolution. One key concern is the surface tension, a parameter that influences the wetting properties of the medium, which is far lower in human gastric fluids than in SGF (35–45 vs. 57 mN/m) (16,17). The sources of surfactants responsible for low surface tension in the fasted gastric fluids have not been conclusively identified; however, reflux of the bile secretions from duodenum as observed in some healthy subjects coupled with the presence of pepsin appear to be the factors most relevant to this phenomenon. To better simulate the wetting conditions in the human stomach in the preprandial state, some adjustments have been made to the design of fasted-state gastric media. One of the early attempts included addition of synthetic surfactants like sodium lauryl sulfate (SLS) or Triton-X[®] 100 into the fasted gastric medium to reduce its surface tension to physiological values (5,18). However, subsequent studies showed that dissolution media prepared using these components overestimated the dissolution of drug products (16). Another key concern is that, when pepsin is used, the level indicated in the SGF described in the USP (3.2 mg/mL) (13) is too high compared with that in the basal gastric pepsin output *in vivo* (the upper limit of pepsin concentration is 0.8 mg/mL in an empty stomach). A third concern is that the pH in the SGF medium is only 1.2, an acidity level that is rarely observed even in young healthy volunteers (especially after the ingestion of a glass of water). pH values of between 1.5 and 2.5 are more the norm in such subjects. In 2005, Vertzoni et al. (16) proposed fasted-state simulated gastric fluid (FaSSGF) as a dissolution medium simulating the preprandial stomach. This medium has a pH of 1.6 and contains 0.1 mg/mL pepsin and low amounts of bile salts and lecithin. The medium has the surface tension close to physiological values (42.6 vs. 35–45 mN/m) (17) and thus appears to be more appropriate than the previous attempts at simulating the fasted stomach contents, in that the reduced surface tension is caused by pepsin, bile salts, and lecithin

TABLE 1 Composition of the Medium to Simulate the Preprandial Stomach—Fasted-State Simulated Gastric Fluid

Sodium taurocholate (μM)	80
Lecithin (μM)	20
Pepsin (mg/mL)	0.1
Sodium chloride (mM)	34.2
Hydrochloric acid	qs pH 1.6
pH	1.6
Osmolality (mOsm/kg)	120.7 ± 2.5

Source: From Ref. 16.

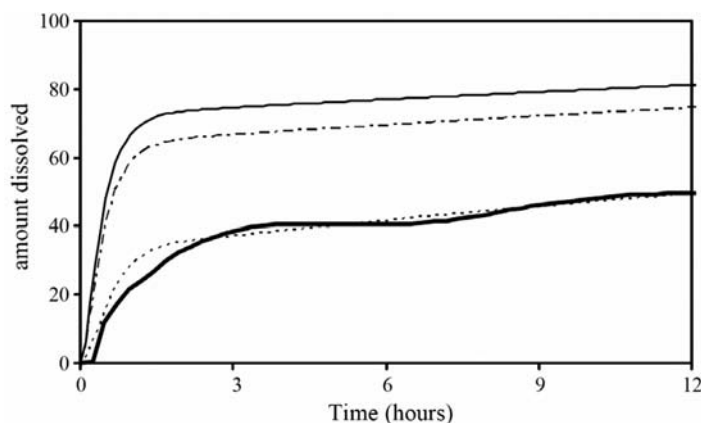


FIGURE 1 Cumulative amount of GR253035X dissolved intralumenally after the administration of one GR253035X tablet (100 mg/tab) in the fasted state versus time (—) and simulated cumulative amounts dissolved intralumenally versus time plots using data in SGF_{SLS} and FaSSIF (—), SGF_{Triton} and FaSSIF (— · —), and FaSSGF and FaSSIF (·····). Abbreviations: SGF, simulated gastric fluid; FaSSIF, fasted-state simulated intestinal fluid; FaSSGF, fasted-state simulated gastric fluid. Source: From Ref. 16.

rather than the synthetic surfactants. The composition of FaSSGF is shown in Table 1.

Good prediction of the oral absorption of a lipophilic model compound (GR253035X—weakly basic compound, $\log P$ 2.8, pK_a 5.1) using FaSSGF as a dissolution medium has been demonstrated (Fig. 1) (16).

Recently, Aburub et al. proposed a revised composition of fasted-state gastric medium (19). This medium contains lower amount of SLS than that proposed by Dressman et al. (1.75 vs. 8.67 mM) (5). The potential advantage of this medium compared with that in biorelevant FaSSGF would be its rather simple composition. Unlike FaSSGF, it does not need to be prepared freshly since there are no biological degradable components, and so storage of the medium over an extended period is possible. The disadvantage lies in the use of SLS at its critical micelle concentration (CMC). On the one hand, this produces a surface tension considerably lower than that of gastric juice (34 vs. 41 mN/m in gastric aspirates and 43 mN/m in FaSSGF), and on the other hand the surface tension is highly dependent on concentration at concentrations below the CMC, which may lead to a high variation in the surface tension with slight variations in concentration.

Fed State

Generally speaking, compared with the fasted state, dissolution of drugs in the fed stomach is usually slow, as shown, for example, by Kelly et al. (20). Retarded disintegration process of the dosage form in the fed stomach, for example, caused by formation of a film around tablets, appears to be responsible for the subsequent slow dissolution of the disintegrated particles (21). This phenomenon was shown *in vitro* using a medium based on a nutritional drink and the results corresponded well with the *in vivo* data from Labradors (21).

However, it is challenging to establish appropriate but easy to work with dissolution media for simulating the fed-state stomach. The first reason is that conditions in the stomach after the meal intake can vary largely, depending on meal type (12,22). The second reason is the different conditions with time after meal ingestion (12,22). In addition, human studies have confirmed that under conditions simulating a bioavailability (BA)/BE study in the fed state, changes in intragastric environment with time are much more pronounced than in the small intestinal milieu (12).

After disintegration, drug particles in the stomach “experience” a changing intragastric environment. Despite this continuously changing environment, it is desirable for practical purposes to have a “global” representative medium that can be used during the drug development process in the *in vitro* tests to compare drug products and/or to estimate food effects.

The use of nutritional liquids for measuring intragastric dissolution was first proposed more than 20 years ago. These include the use of milk (6,23–31) and artificial liquid meals (29,32,33). Although this approach can be theoretically justified for estimating intragastric drug release rates in the fed state, demonstrations of better prediction of absorption after postprandial administration in the literature are lacking (34,35).

Klein et al. proposed a dissolution medium consisting of Ensure[®] Plus and 0.45% pectin to increase the viscosity to physiological values (35) on the basis of the properties of the standard breakfasts used for the evaluation of food effect in pharmacokinetic studies. However, difficulties in drug analysis limit application of this approach. Simpler emulsion systems have been proposed as dissolution media simulating the fed stomach in many studies (17,29,30,32). For example, Luner and VanDer Kamp (17) proposed an emulsion-based medium, fed-state gastric emulsion system (FSGES), to represent the digestion of fat in the stomach. The composition of FSGES is described therein (17). The amount of bile salt contained in this medium (0.5 mM) is questionable, as it is much higher than that contained in the antral aspirates collected in human volunteers [where no bile salts were detected in most postprandial samples (12)] and the values reported in other literature, for example, Ref. 36, 60 μM .

To design a relatively simple approach as an alternative to those mentioned above, ultra-heat treatment (UHT)-milk (3.5% fat) can be used since its composition, in particular, the ratio of carbohydrate to protein to fat, is similar to that observed in the stomach, after administration of meals resembling those administered in BA and BE studies, for example, in meals recommended by the U.S. Department of Health and Human Services, Food and Drug Administration (HHS-FDA) (37) and the European Medicines Evaluation Agency (EMA) (38).

Recently, two different concepts have been proposed to simulate the fed-state gastric conditions. These approaches take into account the swift and substantive changes in the intragastric environment in response to ingestion of a meal.

TABLE 2 Composition of the Media to Simulate the Postprandial Stomach Including FeSSGF

	Early	Middle (FeSSGF)	Late
Sodium chloride (mM)	148	237.02	122.6
Acetic acid (mM)	—	17.12	—
Sodium acetate (mM)	—	29.75	—
o-Phosphoric acid (mM)	—	—	5.5
Sodium dihydrogen phosphate (mM)	—	—	32
Milk:buffer	1:0	1:1	1:3
Hydrochloric acid/sodium hydroxide	qs pH 6.4	qs pH 5	qs pH 3
pH	6.4	5	3
Osmolality (mOsm/kg)	559 ± 10	400 ± 10	300 ± 10
Buffer capacity (mmol/L/pH)	21.33	25	25

Abbreviation: FeSSGF, fed-state simulated gastric fluid.

Source: From Ref. 39.

The first approach is the design of “snapshot” media, which involves the use of a series of milk-based media reflecting the transient changes in environment postprandially, resulting from the gastric secretions and meal emptying process (39). The composition of various gastric snapshot media is presented in Table 2, and the media preparation is described elsewhere (39). Applying this approach, the gastric digestion process is divided into “early,” “middle,” and “late” phases, covering a time frame of approximately 200 minutes. UHT-milk (3.5% fat) is diluted with buffer to simulate the secretions and emptying in the middle (1:1) and late (1:3) phases of meal digestion in the stomach. The pH, osmolality, and buffer capacity of the media are adjusted according to the in vivo human data (12).

These snapshot media can be used as sequential dissolution media in one test series (e.g., using the USP apparatus 3 and 4) when the performance of dosage forms to be evaluated remains in the stomach for an extended period in the fed state (e.g., extended release monolithic dosage forms), and is sensitive to the changing composition of the gastric fluids with time. However, generally speaking, the middle medium represents a global view of most of the physiological changes relating to the meal intake. As such, it can be used to observe food effects in the stomach (compared with FaSSGF) and has been designated as fed-state simulated gastric fluid (FeSSGF) (39). This medium most nearly represents the gastric conditions observed in the 75 to 165 minutes time frame, postprandially. It contains UHT-milk and acetate buffer mixed in equal volumes and has a pH of 5.0. Successful IVIVC has been recently demonstrated by applying FeSSGF to predict the oral absorption of an experimental Roche compound, RZ-50, a poorly soluble weakly acidic drug formulated as a lipid-based dosage form, in dogs (40). In that report, correlations between the in vitro release in FeSSGF using USP apparatus 3 (reciprocating cylinder, Bio-Dis) and the in vivo fraction of drug absorbed were demonstrated by means of level A IVIVC and fitting of dissolution results with the Weibull distribution. Figure 2 shows the comparison of fraction of drug absorbed and fraction of drug dissolved in FeSSGF (Fig. 2A) and FeSSIF (Fig. 2B) by fitting both data sets to Weibull distribution.

In the second approach, the concept of gradual digestion of the meal during the dissolution experiment has been introduced using UHT-milk (3.5% fat) as the initial medium and gradually digesting it by adding physiologically

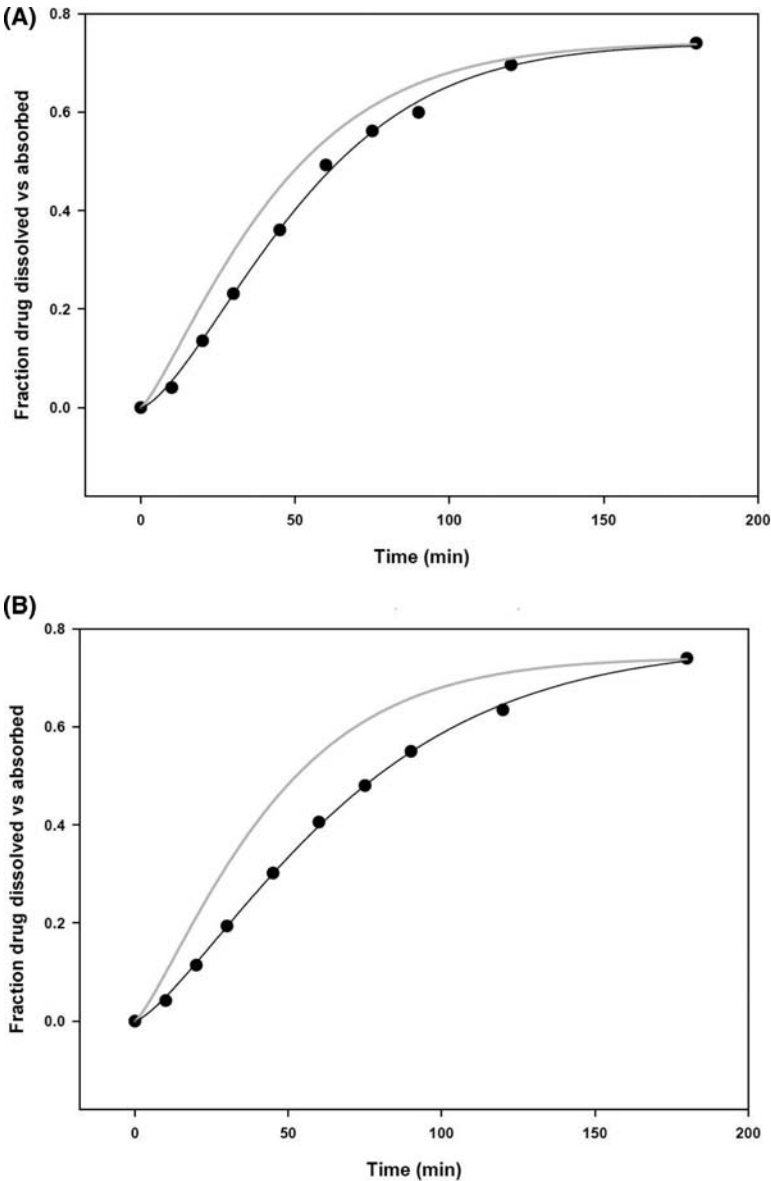


FIGURE 2 Comparison of the fraction absorbed in dogs in the fed state and the fraction dissolved in (A) FeSSGF and (B) FeSSIF using USP apparatus 3 (Bio-Dis) of the Roche model compound, RZ-50. *Abbreviations:* FeSSGF, fed-state simulated gastric fluid; FeSSIF, fed-state simulated intestinal fluid. *Source:* From Ref. 40.

relevant amounts of a hydrochloric solution (1.83 M HCl) containing 1.1 mg of protein (pepsin) per milliliter into the vessel every 15 minutes from time 0 to 90 minutes (41). Applying this procedure, the concentration of pepsin in the medium increases gradually from 0 to 61.6 $\mu\text{g/mL}$, and of hydrochloric acid

from 0 to 102.5 mM over the first 90 minutes. This approach has been shown to be useful in forecasting the effects of intragastric residence on dosage form performance (42). An impression of how the dissolution profile could be affected by digestion in stomach can be obtained from relevant dissolution data for two lipophilic compounds, troglitazone (weak acid, pK_{a1} 6.1, pK_{a2} 12.0, $\log P$ 2.7, RomozinTM 200-mg tablets, GlaxoSmithKline, U.K.) (Fig. 3A), and GR253035X (weak base, pK_a 5.1, $\log P$ 2.8, 100-mg tablets) (Fig. 3B).

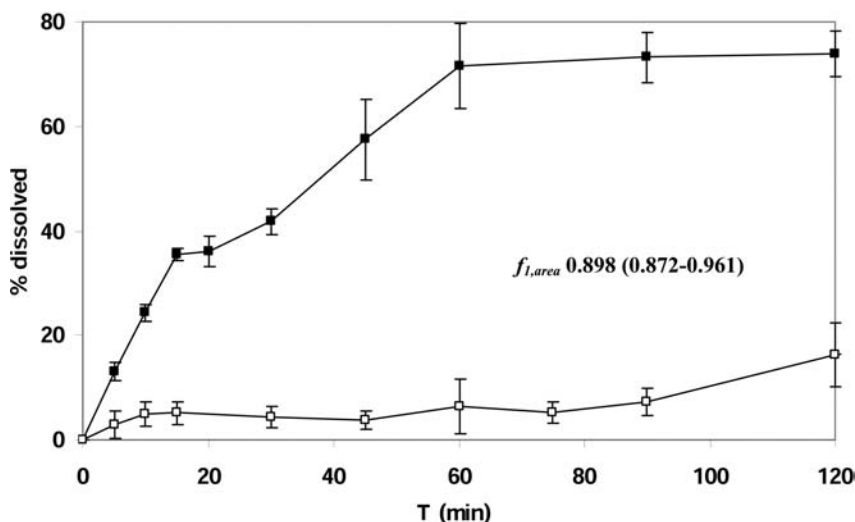
Data from experiments in undigested milk were compared with those in buffers with pH similar to that of milk and in digested milk. For both drugs, the percentage dissolved in milk (pH 6.6) is much higher than the percentage dissolved in buffer with similar pH (43). The effect of digestion on total drug dissolved was assessed by using the value of difference factor, $f_{1,area}$ (44). It is interesting to note that both the gradual decrease of pH and presence of solubilizing proteins affect the dissolution profiles of the model compounds significantly, but in quite different ways (Fig. 3). Recently, it has been suggested that for the simulation of intragastric release profile, simulation of intragastric lipolysis might also be important (45). Simulation of gastric lipolysis can be achieved by adding two portions of lipase RN (at 0 and 90 minutes after the beginning of the dissolution experiment) to maintain mean lipase activity levels between 20 and 50 U/mL (45). Data collected with an HPMC extended-release tablet formulation of felodipine (lipophilic and non-ionizable compound) were close to those observed in vivo in the fed stomach, only if intragastric lipolysis was simulated in addition to the protein digestion (Fig. 4). It would be interesting to assess the usefulness of the gradual digestion approach in the evaluation of other drugs and dosage forms, especially lipid-based IR dosage forms, on the basis of the prediction of intragastric release data in humans.

Dissolution Media to Forecast Dosage Form Performance in the Small Intestine

The small intestine, especially the proximal part, represents the main site of drug absorption in the GI tract. Additionally, for weakly acidic compounds and for neutral and basic compounds that are lipophilic, this region is also important for the dissolution process. Similar to the stomach, but with somewhat less variability, the environment in the proximal small intestine changes considerably after meal intake. Changes include increases in secretions of bile and pancreatic juice as well as appearance of digestive products, all of which can impact solubility and dissolution of drugs.

Two biorelevant media simulating the luminal conditions in the proximal small intestine in the pre- and postprandial states, FaSSIF and FeSSIF, were introduced in 1998 (5) and then applied widely in the pharmaceutical arena (6,31,34). Since then, the media compositions have been either simplified to reduce cost of the experiments and to serve practical purposes and/or modified to better predict the dosage form behavior in vivo (9,47). Nevertheless, the various modifications have some drawbacks. For instance, on the one hand, the simplified media, containing synthetic surfactants, while easy to prepare and inexpensive, can be only used to replace bile components on an empirical basis, since there is no universal factor relating solubility and dissolution enhancement by synthetic surfactants to bile components that can be

(A)



(B)

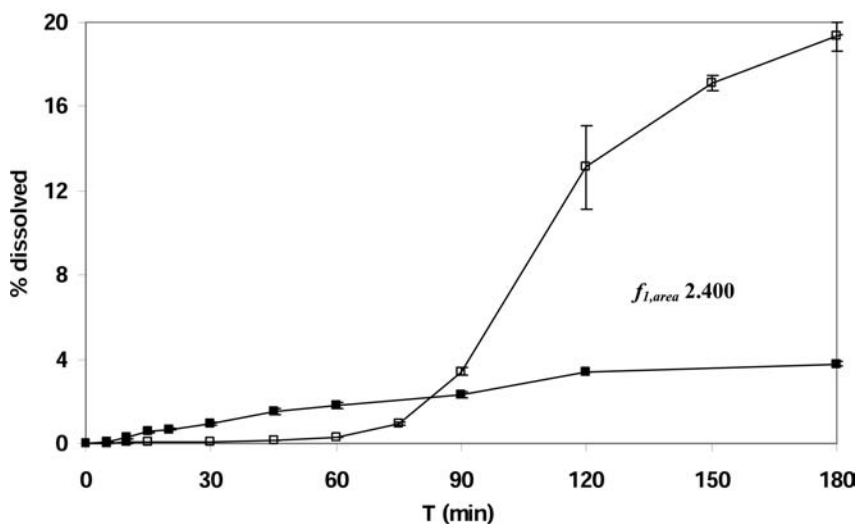


FIGURE 3 Mean $\pm$ SD ($n = 3$) data for the cumulative percent dissolved for (A) RomozinTM and (B) GR253035X tablets, collected with the rotating paddle apparatus (100 rpm) using 500 mL of UHT-milk (3.5% fat) (■), and using 500 mL of UHT-milk (3.5% fat) at which 4 mL of 1.83 M HCl containing 1.1 mg/mL pepsin from hog pancreas were added every 15 minutes for 90 minutes (□). Source: From Ref. 43.

applied for all compounds (47). On the other hand, some modifications like the use of crude bile salts instead of pure sodium taurocholate in the bio-relevant media can lead to problems with standardization of the media composition and sample analysis (9).

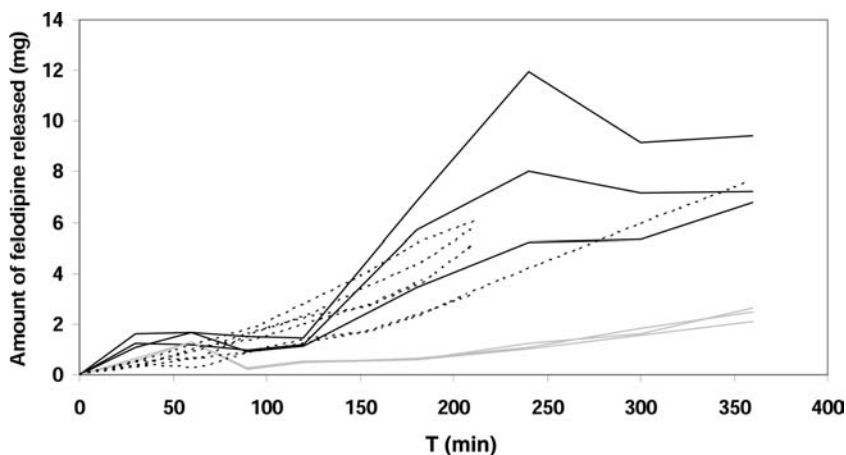


FIGURE 4 Individual cumulative amounts of felodipine released in milk digested with hydrochloric acid solution of pepsin (*grey continuous lines*, $n = 3$), in UHT-milk (3.5% fat) digested with hydrochloric acid solution of pepsin and in presence of lipase RN (*black continuous lines*, $n = 3$), and in the stomach of healthy volunteers *in vivo* (46) (*dotted lines*, $n = 6$). *Source:* From Ref. 45.

Recently, Jantratid et al. (39) have introduced a core group of four biorelevant dissolution media simulating the pre- and postprandial states in the stomach and proximal small intestine. These media include the updated version of FaSSIF and FeSSIF. The media compositions were designed by considering the dissolution and solubility enhancing components from the natural GI juices as well as in the meal digestion products. These dissolution media can be used to serve the purposes of biorelevant dissolution testing and are detailed below.

Fasted State

The compendial medium that has been widely used to represent small intestinal conditions over the years is simulated intestinal fluid (SIF) (13–15). The current version has a pH of 6.8 and contains pancreatin. Although the pH of SIF was revised from 7.5 to 6.8 in 1996, to be closer to the physiological values (48), the properties of SIF are still not a one-to-one copy of the *in vivo* conditions in the small intestine. To simulate the fasted-state proximal small intestinal milieu, in 1998, Dressman et al. (5) and Galia et al. (6) proposed and evaluated FaSSIF as a biorelevant medium. The medium composition is demonstrated in Table 3.

TABLE 3 Composition of the Medium to Simulate the Preprandial Small Intestine—Fasted-State Simulated Intestinal Fluid

Sodium taurocholate (mM)	3
Lecithin (mM)	0.75
Sodium hydroxide (mM)	8.7
Dibasic sodium phosphate (mM)	28.65
Sodium chloride (mM)	105.85
pH	6.5
Osmolality (mOsm/kg)	270 ± 10
Buffer capacity (mmol/L/pH)	10

Source: From Ref. 5.

TABLE 4 Composition of the Medium to Simulate the Preprandial Small Intestine—Fasted-State Simulated Intestinal Fluid, Updated Version (FaSSIF-V2)

Sodium taurocholate (mM)	3
Lecithin (mM)	0.2
Maleic acid (mM)	19.12
Sodium hydroxide (mM)	34.8
Sodium chloride (mM)	68.62
pH	6.5
Osmolality (mOsm/kg)	180 ± 10
Buffer capacity (mmol/L/pH)	10

Source: From Ref. 39.

Sodium taurocholate and lecithin presented in the recipe reflect the basal bile secretions in the small intestine preprandially.

Recently, Jantratid et al. (39) have updated the composition of FaSSIF, which will be referred to as FaSSIF-V2. According to the *in vivo* data summarized by Porter et al. (49), only minor changes to the previous composition, FaSSIF, are required; the amount of lecithin is decreased from 0.75 to 0.2 mM in the updated version. The composition of FaSSIF-V2 is shown in Table 4.

The pH and buffer capacity are maintained in FaSSIF-V2 as for FaSSIF. The osmolality is decreased to match the *in vivo* values. Maleate buffer is used as a composition of FaSSIF-V2 instead of phosphate buffer in FaSSIF because it can be used as a component in both the fasted- and fed-state intestinal media without exceeding the physiologically relevant osmolality. Using physiological buffer as observed in the small intestine, that is, bicarbonate buffer, although proposed as a component of biorelevant media (50), is discouraged because (i) it is relatively difficult to work with this buffer as continuous supply of carbon dioxide is required and (ii) substituting phosphate with bicarbonate in presence of bile salt and lecithin does not improve the prediction of *in vivo* performance (51).

Fed State

After meal intake, conditions in the small intestine deviate from the fasted state quite considerably; however, after the initial rise in bile concentration, the changes in the small intestinal fluid composition over time are not as rapid or as far-reaching as in the fed stomach. Again, simple aqueous buffers cannot be used to represent these conditions, and analogous to FaSSIF, Dressman et al. (5) and Galia et al. (6) proposed FeSSIF as a dissolution medium simulating the postprandial small intestine. Wide applications of the medium (together with FaSSIF) have been reported in the literature (31,34,52,53). Composition of FeSSIF is described in Table 5.

Jantratid et al. (39) have recently revised the composition of FeSSIF. As for the fed-state gastric media, the concept of snapshot media was also applied to the design of small intestinal media. Early, middle, and late phases represent different time frames of the digestion process in the small intestine. From these snapshot media a global medium was further designed. The medium represents a global level of bile secretions in the postprandial small intestine and additionally contains some lipolysis products. The major deviations from the previous composition, FeSSIF, include the lower amount of bile components in FeSSIF-V2, the updated version. This is, at least, partly compensated for in terms of solubilization capacity by the addition of the lipolysis products, glyceryl monooleate and

TABLE 5 Composition of the Medium to Simulate the Postprandial Small Intestine—Fed-State Simulated Intestinal Fluid

Sodium taurocholate (mM)	15
Lecithin (mM)	3.75
Acetic acid (mM)	144.05
Sodium hydroxide (mM)	101
Sodium chloride (mM)	203.18
pH	5.0
Osmolality (mOsm/kg)	670 ± 10
Buffer capacity (mmol/L/pH)	76

Source: From Ref. 5.

TABLE 6 Composition of the Media to Simulate the Postprandial Small Intestine Including FeSSIF-V2

	Early	Middle	Late	FeSSIF-V2
Sodium taurocholate (mM)	10	7.5	4.5	10
Lecithin (mM)	3	2	0.5	2
Glyceryl monooleate (mM)	6.5	5	1	5
Sodium oleate (mM)	40	30	0.8	0.8
Maleic acid (mM)	28.6	44	58.09	55.02
Sodium hydroxide (mM)	52.5	65.3	72	81.65
Sodium chloride (mM)	145.2	122.8	51	125.5
pH	6.5	5.8	5.4	5.8
Osmolality (mOsm/kg)	400 ± 10	390 ± 10	240 ± 10	390 ± 10
Buffer capacity (mmol/L/pH)	25	25	15	25

Abbreviation: FeSSIF-V2, fed-state simulated intestinal fluid, updated version.

Source: From Ref. 39.

sodium oleate. Further, the pH is increased from 5.0 to 5.8 to better match the physiologically observed values (49). The buffer capacity and osmolality are lower in FeSSIF-V2 than in FeSSIF. FeSSIF-V2 can be used to generally estimate the dissolution of dosage forms in the postprandial small intestine, whereas the snapshot small intestinal media are more useful when questions about dissolution/release in specific time frames after meal ingestion are to be addressed. Table 6 shows the composition of the snapshot media and of FeSSIF-V2.

Applications of FaSSIF-V2 and FeSSIF-V2 with respect to the *in vivo* predictiveness have been shown recently (54). The updated media predicted correctly that the oral absorption of IR glibenclamide tablets in the fasted and fed states is not significantly different. This result is in contrast to estimates obtained using the earlier media compositions, with which apparent differences in the dissolution profiles between the prandial states were obtained (Fig. 5) (53). Since most *in vivo* studies have not shown food effects with glibenclamide, it appears that the new version media may offer some advantages over the previous compositions.

SIMULATION OF INTRALUMENAL HYDRODYNAMICS

Perhaps the first attempt to develop a complete biorelevant *in vitro* setup dates back to 1948 (10). Although the chosen *in vitro* conditions would be questioned today, the early attempt took into consideration the amount and quality of saliva

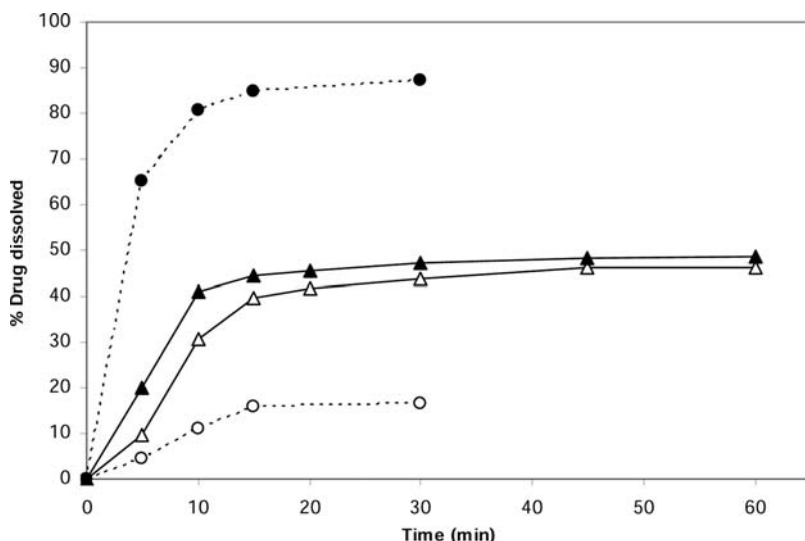


FIGURE 5 Dissolution profile comparison of glibenclamide tablets (Euglucon N[®]) in FaSSIF (●) and FeSSIF (○) (both dotted lines), and in FaSSIF-V2 (▲) and FeSSIF-V2 (△) (both continuous lines). Abbreviations: FaSSIF, fasted-state simulated intestinal fluid; FeSSIF, fed-state simulated intestinal fluid. Source: From Ref. 54.

in the mouth, the acidity and volume of gastric juice at the time of swallowing, the amount of peristaltic movements, and the hydrostatic pressure present during peristalsis (Fig. 6). In addition, the gastric emptying process was simulated (Fig. 6). That setup proved to be useful for predicting in vivo disintegration times, as monitored by observing in vivo disintegration with radiopaque tablets (10). However, the approach was never evaluated for predictions of intraluminal dissolution rates.

Although dissolution in biorelevant media is increasing our ability to forecast mean plasma levels or the average fraction of drug absorbed (34,55,56), the hydrodynamics employed in the in vitro dissolution setups are still based on compendial apparatus. Most frequently used are the USP apparatus 2 (paddle assembly), apparatus 3 (reciprocating cylinder), and apparatus 4 (flow-through cell) (13). An assessment of the hydrodynamics when using these apparatus can be based on the (dimensionless) Reynolds numbers. The Reynolds number is used to characterize the laminar-turbulent transition, and is commonly described as the ratio of momentum forces to viscous forces in a moving fluid (57). Reynolds numbers for the bulk flow vary from less than 30 (when using the USP apparatus 4) (58) to more than 2000 (when using the USP apparatus 2) (57). There are currently no data for USP apparatus 3. Since the Reynolds number characterizing laminar-turbulent transition for bulk flow, in a pipe that behaves in a hydraulically smooth manner, is about 2300 (57), hydrodynamics in the in vitro setups used can create bulk flow conditions in both the laminar and turbulent regions. It would be interesting to know how intraluminal hydrodynamics compare to those in the compendial in vitro setups. However, it is difficult to pin down hydrodynamics in the upper GI tract, since on the one

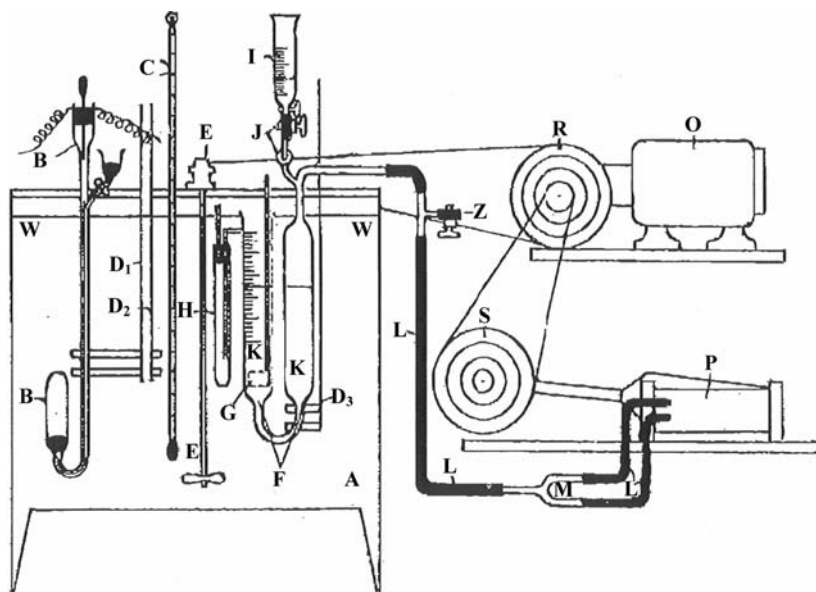


FIGURE 6 Assembly for physiological tablet disintegration test. (A) thermostatically controlled water bath, (B) thermostat, (C) thermometer, (D₁, D₂, D₃) supporting rods and clamps, (E) stippler, (Z) compression adjustment, (F) vessel artificial stomach, (G) plastic tablet container, (H) collecting tube, (I) 100 mL burette, (J) drop meter, (K) artificial stomach juice (100 mL), (L) rubber tubes, (M) "Y" cannula, (O) electric motor, (P) oscillating respiration pump, (R) electric motor wheel, (S) pump wheel, (W) water level. *Source:* From Ref. 10.

hand both flow rates and viscosity of luminal contents vary dramatically (57) and, on the other hand, the intestine does not behave like a conventional pipe, but rather the gut wall contracts.

The need for better simulation of *in vivo* hydrodynamics, especially in the fasted state, has been recently shown with danazol as the model compound (37). Simulation of the average plasma profile in the fasted state was greatly improved when biorelevant media were used, but the best simulation was obtained only when the (compendial) flow-through apparatus was operated at nonphysiologically relevant flow rates (32 mL/min) (55).

Because of both the complexity and variability of intraluminal motility (59) and limited human data on the intraluminal volumes, flow rates, and bidirectional water flux through the intestinal wall, no major progress on simulating *in vivo* hydrodynamics was made before the end of the 20th century, apart perhaps from a few attempts to develop artificial GI systems for the study of digestion of foods (60,61). A quantitative simulation of intraluminal hydrodynamics is not a primary goal of these artificial systems, nor have they been systematically assessed in terms of the ability to predict drug absorption from IR dosage forms (62).

Information with respect to the intraluminal hydrodynamics is still limited, but in recent years some relevant studies have been conducted both for gastric emptying and small intestinal passage. Using concurrent magnetic resonance imaging (MRI) and high-resolution manometry, Indireskumar et al.

showed that physiological coordination between pyloric and antral contractile activity is necessary for transpyloric flow of nonnutrient saline to occur (63). Pallotta et al. evaluated the patterns of antral contractility and pyloric opening and closing in relation to transpyloric flow of nutrient liquid meal using ultrasound images of the antro-pyloro-duodenal tract in healthy volunteers (64). It was shown that the final passage of contents from the stomach to the duodenum is the result of one or more episodes of uni- or bidirectional transpyloric flow, which are regulated by several motor events. A crucial regulator of transpyloric flow appeared to be the spatiotemporal relation between antral contractions and pyloric closure rather than the contractile events per se (64). Cassilly et al. showed (using SmartPill GI monitoring capsule) that a nondigestible solid empties from the stomach with the occurrence of high-amplitude antral contractions (65). In most cases, the nondigestible solid emptying occurs with the return of the phase III of the migrating motor complex of the fasting period and, in some cases, the emptying is occurred with isolated antral contractions (65).

Computer models based on images obtained with MRI and wall movements of the stomach have also been used to predict the transport of fluid and solids along the GI tract (66). It has been demonstrated that fluid is not homogeneously distributed along the gut, which likely contributes to the individual variability of drug absorption (66). Intestinal fluid is located in pockets of variable volume, which are irregularly scattered along the intestine and solid dosage forms are not consistently in contact with these fluid pockets (66). Transport of fluid and solids through the ileocecal valve is initiated by a meal-induced gastroileocecal reflex. As a result of these observations, attempts to design in vitro dissolution setups that better take into account the intraluminal hydrodynamics started to appear in the literature in the last few years.

Abrahamsson et al. (67) designed an in vitro apparatus that can simulate the in vivo range of surface shear stresses relevant for the human stomach under the fed conditions. This apparatus consists of a rotating beaker with the tablet fixed on a steel wire. The beaker is glued to the centrally placed rod at the bottom so that when the rod is rotated at a fixed revolution rate the beaker also rotates at the same rate. Shear force effects on drug release from matrix tablets relevant for fed state could be predicted with this setup (67), but direct correlation with intragastric release data has yet to be shown. This apparatus is similar to another apparatus that utilizes the USP apparatus 2 dissolution tester with the tablet fixed on a steel wire (67). With the latter apparatus, adequate prediction of the intraluminal behavior of modified-release (MR) tablets has been achieved (45,68).

In 2006, Burke et al. (69) created an apparatus that simulates the conditions in the GI tract by applying forces to the dosage form. The frequency, duration, and amount of force or compression that are applied to the dosage form can be controlled and preferably varied. This is done by a programmable logic computer. The device has a housing, an impeller, a sampler, and a force application system (Fig. 7). The force application system is mounted or connected with the housing of the analysis device and has a dosage form housing and a force imparting mechanism (Fig. 7). The dosage form housing is a cylindrical chamber having a mesh screen along the bottom of the chamber. The force imparting mechanism is a piston with a number of holes formed there through, which allow for flow of the aqueous solution into and through the chamber (Fig. 7). The impeller provides motion to the aqueous solution to distribute the active

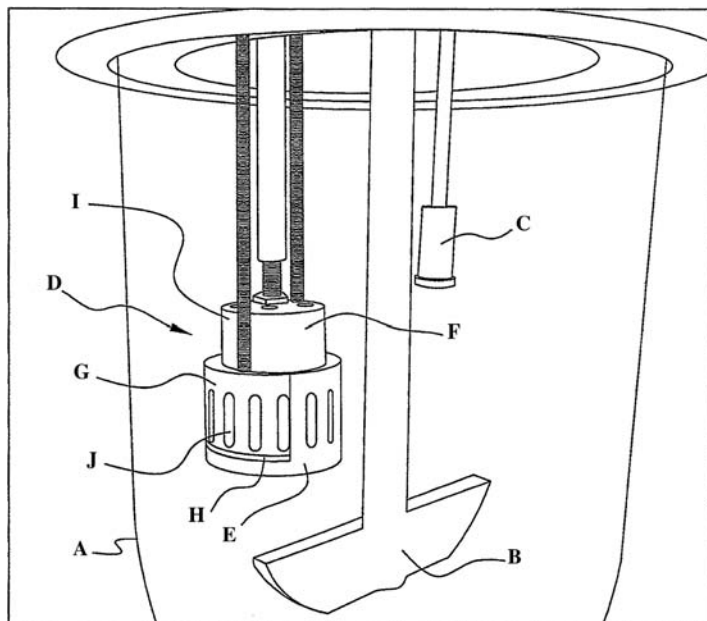


FIGURE 7 A perspective view of the device of the patent with the impeller and the sampler. The device has a housing (A), an impeller (B), a sampler (C), and a force application system (D). The force application system has a dosage form housing (E) and a force imparting mechanism (F). The dosage form housing is a cylindrical chamber (G) having a mesh screen (H) along the bottom of the chamber. The force imparting mechanism (F) is a piston (I) with a number of holes (J) formed there through. *Source:* From Ref. 69.

agent in the solution and to further simulate the conditions of the GI tract. The sampler obtains samples of the aqueous solution to determine the amount of active agent that has been released by the dosage form.

Although the superiority of this apparatus over the conventional release apparatus to predict intraluminal drug release has been shown with only a few examples to date (69), it certainly warrants further evaluation.

Very recently, a dissolution test device has been proposed with which simulation of the physical stress conditions present during GI passage of dosage forms can be simulated (70). This device seems to enable simulation of the three main physical stress factors that occur during GI transit: pressure forces exerted by gut wall motility, shear forces generated during propagation, and loss of water contact when dosage form is located in an intestinal air pocket (Fig. 8). To date, this approach has been successfully applied for the prediction of irregular plasma profiles after administration of a monolithic extended release product (70). It would be interesting to evaluate its usefulness in similar predictions after administration of IR formulations.

In summary, although simulations of luminal hydrodynamics have started to appear in the literature in recent years, it is too early to comment which device will prove to be the most useful in biorelevant dissolution testing of IR dosage forms.

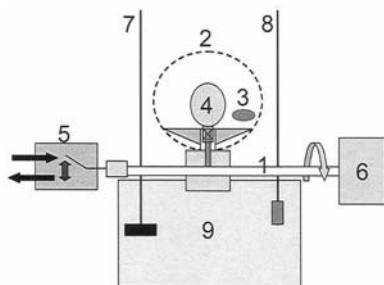


FIGURE 8 Schematic representation of the dissolution stress test device. (1) Central axis ($\varnothing$ 8 mm), (2) chamber ($\varnothing$ 35 mm mesh size 0.5 mm, wire 0.1 mm), (3) dosage form, (4) inflatable balloon, (5) solenoid valves, (6) stepping motor, (7) stirrer (paddle 15×35 mm²), (8) sampling, and (9) standard vessel. Source: From Ref. 70.

SUMMARY

Dissolution is considered to be rate limiting to absorption of poorly soluble compounds. As a result, it is appropriate to apply biorelevant dissolution test conditions to these products to obtain meaningful predictions of their in vivo performance.

Over the last decade much progress has been made toward generating and improving biorelevant dissolution media that can be used for predicting oral drug absorption including the food effect. The media compositions of the current version come closer to the GI fluids in vivo. Nevertheless, it is apparent that further fine-tuning of their compositions may be required.

Even though hydrodynamics can obviously play a key role in drug release and dissolution, they have been accorded little attention until recently. Several apparatus setups have now been proposed and results indicate that they may show several advantages over existing compendial apparatus.

Especially in the context of Quality by Design (QbD), it will be important to have biorelevant tests at our fingertips for linking composition and manufacturing parameters to therapeutic effectiveness in the future. Combining the advantages of biorelevant media with better simulation of luminal hydrodynamics represents the way forward to achieving QbD objectives and enabling better predictions of in vivo drug product performance.

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Dissolution Testing to Forecast the In Vivo Performance of MR Formulations

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INTRODUCTION

Modified-release (MR) dosage forms have represented a broad segment of research and development in the pharmaceutical industry for many years. Incorporating an existing drug into a new drug delivery system can significantly improve its performance in terms of efficacy, safety, and patient compliance. Oral ingestion is by far the most popular route of drug administration, providing a convenient method to release drugs in a controlled and predetermined fashion and/or target to selective sites in the gastrointestinal (GI) tract. However, neither in the scientific literature nor in current pharmacopoeias can a harmonized definition of modified release for oral delivery be found.

As a fundamental, technological distinction, MR dosage forms can be categorized into single-unit dosage forms (e.g., matrix tablets), consisting of one discrete entity that contains one dose of the drug and is intended to be administered individually, and multiple-unit dosage forms consisting of many small discrete units (e.g., pellets), which together provide the overall MR profile.

With the various types of oral MR dosage forms available, it is a challenge to accurately predict their in vivo behavior. Ideally, drug release from oral MR formulations is dependent exclusively on the dosage form, with little or no influence from the intrinsic properties of the drug or the conditions prevailing in the GI tract. Experience has shown, however, that this cannot be generally assumed to be the case. Substitution of one MR formulation by another or administering the same formulation under varying dosing conditions (e.g., fasted vs. fed state) can result in unexpected effects. Unwanted effects that have been described in the literature during the last decades range from “dose dumping” to subtherapeutic plasma levels. As these unwanted side effects may result in severe risks for the patients, it would be highly desirable to be able to forecast the in vivo release rates under various dosing conditions using in vitro data.

The in vivo performance of oral MR dosage forms is determined by the interplay of three major variables (i) the physicochemical properties of the drug, (ii) the composition and characteristics of the dosage form, and (iii) anatomical and physiological conditions. As a result, to accurately predict the in vivo drug-release behavior from an MR dosage form based on in vitro release rates, it is crucial to first classify the MR dosage form in terms of drug substance, excipient composition, and method of manufacture, as well as to take into account the proposed dosing conditions (e.g., before or after a meal), and then to design an adequate release test system that is relevant to the in vivo conditions of release. To create a release test system that can predict whether the MR dosage form meets its in vivo release profile goals, it is particularly important to adequately simulate all parameters that may affect drug release from MR dosage forms in the dissolution experiment.

DISSOLUTION TEST METHODS

Official Test Methods

Dissolution test devices for testing solid oral dosage forms are currently described in various international pharmacopoeias. The largest number of official methods can be found in the U.S. Pharmacopoeia which contains many monographs specifying dissolution conditions for various drug products (i.e., “monographed dissolution tests”). Because of the importance dissolution testing has assumed in the last few decades, various generalized guidelines that provide information and recommendations on the development of dissolution test methodology, set dissolution specifications, and describe the regulatory applications of dissolution testing have also been developed (1–4). However, all official dissolution methodologies used to characterize drug release from oral MR dosage forms are based on compendial dissolution apparatus combined with simple aqueous dissolution media. So while they are generally useful for quality control, they do not reflect many of the aspects of GI physiology. Nonmonographed dissolution methods for MR dosage forms can also be developed on a case-by-case basis. The primary focus of these methods in most cases is to achieve an in vitro–in vivo correlation (IVIVC). Nevertheless, as with the official methods, most of the nonmonographed methods developed to date make no attempt to closely reflect physiological conditions in the GI tract and therefore need to be optimized to increase their ability to predict the in vivo release behavior of the formulation.

Because of the various types of MR formulations that are available, it may be unrealistic to expect that a simple and unique dissolution method can be developed, which would be universally applicable. However, it is possible to identify an array of methods that can facilitate prediction of in vivo performance for specific groups of MR dosage forms.

Objectives for Improving the Biorelevance of Dissolution Methods

Ideally, a predictive dissolution method for MR formulations should be as simple as possible, reliable, and reproducible and should make it possible to discriminate appropriately between different degrees of product performance (5). However, to achieve adequate predictability of the in vivo release behavior of MR dosage forms by use of in vitro dissolution data, physicochemical properties of the drug and its formulation as well as the relevant physiological conditions have to be considered in equal measure. A so-called biorelevant dissolution system should therefore be able to simulate conditions in the human GI tract in terms of dosing conditions and therapeutic objective. Test conditions should reflect the GI conditions that are relevant to drug release from the dosage form to be tested. Special attention should be paid to

- pH conditions,
- other key aspects of the composition of the GI contents (osmolality, ionic strength, viscosity, surface tension, etc.),
- volume of the GI contents,
- motility patterns,
- passage times/residence times, and
- dosing conditions.

Inherent in the above list is also the influence of food ingestion on drug release.

To fulfill these requirements, it is necessary to use both test equipment that can simulate the dosage form passage through various sections of the GI tract and test media that reflect relevant conditions in the GI tract. Official methods and regulations predominantly prescribe the use of USP apparatus 1 (basket) and 2 (paddle) combined with aqueous buffer media of various pHs. But neither apparatus can simulate passage of an MR dosage form through different sections of the GI tract in a meaningful way as both are closed systems that consist of a single vessel for each dosage form and are mostly operated with a fixed volume of a single medium. Simple aqueous buffer media cannot be appropriate for every type of MR dosage form, because they neither reflect the changing physiological environment with passage through the GI tract nor represent the composition of GI fluids after meal intake. Thus, particular attention must be given to the design of appropriate biorelevant dissolution media for the different types of MR dosage forms.

Over the last years, attempts have been made to simulate different physiological parameters relevant for drug release in the GI tract by developing new types of dissolution media (6–8), using more sophisticated apparatus, for example, flow-through apparatus (9,10), or combinations of these innovations (11,12). However, most of these methods address either a few selected aspects that are known to be important for simulating GI conditions and/or are mainly used for immediate-release (IR) dosage forms. Hence, it is a logical next step to develop dissolution methods for MR dosage forms that enable simulation of GI passage following administration in either the fasted or the fed state and which can be directly applied to generation of IVIVCs.

Test Equipment

In 1991 the USP 22 adopted the reciprocating cylinder apparatus (USP apparatus 3, BioDis[®]) as an alternative to basket and paddle apparatus for drug-release testing (Figs. 1 and 2). This apparatus is the most attractive for the study of MR



FIGURE 1 USP apparatus 3 (BioDis[®])—complete setup. Abbreviation: USP, United States Pharmacopoeia. Source: Courtesy of Erweka GmbH.

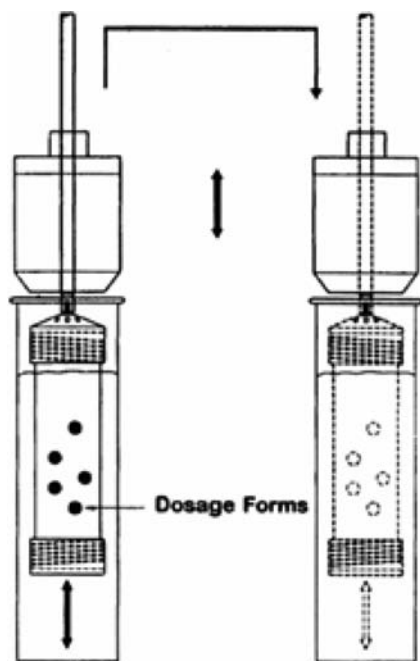


FIGURE 2 USP apparatus 3: glass cylinder moving from one vessel to another. *Source:* Courtesy of Hanson Corp.

formulations, as it offers many advantages in terms of mimicking the changes in physicochemical conditions and mechanical forces experienced by products in the GI tract (13).

To create a biorelevant yet easy to operate dissolution setup, USP apparatus 3 offers several clear advantages over apparatus 1 and 2. Because of numerous programmable options, it is possible to simulate human GI passage in terms of passage times, hydrodynamic conditions (using various combinations of dip rate and mesh size), and possible carryover effects from one section to another. Combining this apparatus with appropriate dissolution media, the ability to predict the profile of drug release from MR dosage forms *in vivo* can be improved.

A further apparatus that appears to be appropriate for this purpose is USP apparatus 4, the flow-through cell (Fig. 3). This apparatus also offers the possibility of varying the composition of media and the flow rates during the test



FIGURE 3 USP apparatus 4: open loop system. *Source:* Courtesy of Erweka GmbH.

and can be used as either a closed or an open system. This is an advantage over the BioDis apparatus, which is restricted to the closed, fixed-volume mode. A further advantage of using the flow-through apparatus is the possibility of continuous online UV detection of the amount of drug released from the dosage form using a simple spectrophotometer. However, this advantage is no longer relevant when biorelevant media are used, since light scattering due to, for example, the presence of mixed micelles of bile salts and lecithin or other emulsifying agents usually results in a need for chromatographic analysis. Moreover, if biorelevant dissolution media are to be used, USP apparatus 3 seems to be more economical for this objective, since most flow-through experiments require huge volumes of media if run in the open-system mode. As biorelevant media are very expensive and relatively tedious to prepare, a large consumption of media does not meet the objectives of test design. Further, various filters and tubes that belong to the setup of the flow-through apparatus tend to plug frequently, especially when using biorelevant media to simulate passage through the fed stomach. A further disadvantage of the flow-through cell is that, within a run, the hydrodynamics can only be adjusted by altering the flow rate. The use of glass beads in the cell, which can also be used to modify hydrodynamics, must be decided prior to beginning the test and cannot be changed during a run. Thus, the BioDis apparatus is much more flexible in terms of hydrodynamic adjustments during the course of a test.

All arguments taken together, USP apparatus 3 appears to be the most promising apparatus for biorelevant dissolution testing of MR formulations as this setup offers the possibility of simulating the passage through the human GI tract using different media, residence times, and hydrodynamic conditions. It is also advantageous in terms of robustness and economics.

Dissolution Media

It was pointed out that drug bioavailability from an oral dosage form depends only partly on the properties of the active substance and the excipients, and that the dosing conditions, that is the timing of administration and any coadministered fluids or food, are additional important criteria. In particular, the biopharmaceutical parameters, for example the wettability/swellability, mechanical stability, and dissolution rate of a formulation can show high variability, depending on food intake. Hence, food-induced changes in the GI physiology have to be addressed in both in vivo and in vitro experiments, if one hopes to predict the influence of food on formulation performance. Thus, the choice of appropriate media for the in vitro tests is crucial to the ability to correctly forecast food effects in pharmacokinetic studies.

The official media used to determine drug-release behavior from MR dosage forms are generally the same as those for IR dosage forms. However, a single medium is likely not to result in dissolution profiles that are predictive for in vivo release of the dosage form. Even methods applying combinations of a gastric and an intestinal medium to simulate dosage form transfer from the stomach into the small intestine are too simple for this purpose, and in particular such methods are not useful to examine the impact of food intake on dosage form performance.

As the main differences in GI physiology between the fasted and fed state occur in the upper GI tract where most types of MR dosage forms start to release

the active drug, it is particularly important to simulate these conditions in vitro. On the basis of these considerations, several biorelevant media to simulate conditions in the stomach and small intestine before and after meals have been developed over the last decade.

Media to Simulate the Upper GI Tract in the Fasted State

Simulated gastric fluids. The traditional medium to simulate gastric conditions in the fasted state has been simulated gastric fluid (SGF) of the USP (similar fluids are also described in other compendia). This medium contains hydrochloric acid and sodium chloride, as well as pepsin and water, and has a pH of 1.2. Although the medium addresses many of the qualities of gastric juice, there are some aspects that could be optimized. For example, most studies of gastric pH, even in young healthy volunteers, indicate that gastric pH usually lies in the range 1.5 to 2.5, with an across-the-board average of about 1.6 to 1.8. Therefore for some drugs, particularly very poorly soluble weak bases, the dissolution results in SGF are likely to overestimate the in vivo rate. A further deviation from gastric physiology is the pepsin concentration, which is very high compared to that observed in gastric juice aspirated under fasted-state conditions. On the other hand, no attempt is made to simulate the surface tension of the gastric fluid. This has been repeatedly measured as lying in the 35 to 50 mN/m range. The official SGF (without pepsin) in contrast has a surface tension of 70 mN/m.

To screen for reliable and reproducible performance of dosage forms under gastric conditions, fasted-state simulated gastric fluid (FaSSGF), a gastric medium which more adequately reflects physiological conditions and additionally takes into account the reduced surface tension observed in the fasted-state stomach, was developed (14). The composition of a slightly modified FaSSGF used for the experiments presented in this chapter is shown in Table 1.

Simulated intestinal fluids. A frequently used medium for the simulation of small intestinal (SI) conditions in the fasted state is simulated intestinal fluid (SIF) pH 6.8 of the USP. This medium represents the average pH conditions in the jejunum; however, it does not adequately reflect all aspects of physiological conditions in the small intestine and therefore dissolution rates of drugs in SIF may not provide good predictions of the dissolution of drugs in vivo. In addition to pH, further important physiological factors not adequately addressed with SIF are buffer capacity, bile and pancreatic secretion, surface tension, osmolality,

TABLE 1 Sample Composition for Simulating Fasted-State Gastric Conditions

FaSSGF pH 1.8 ^a	
Sodium chloride	34.2 mM
Sodium taurocholate	80 μ M
Lecithin	20 μ M
Hydrochloric acid conc.	3 g
Deionized water	ad
pH	1/8
Osmolality (mOsmol/kg)	120.7 + 2.5
Surface tension (mN/m)	42.6

^aOriginal composition has a pH of 1.6 and contains 0.1 mg/mL pepsin.

Abbreviations: FaSSGF, fasted-state simulated gastric fluid; ad, up to; qs, a sufficient quantity.

TABLE 2 Composition of the Biorelevant Medium Used to Simulate Fasted-State Conditions in the Small Intestine

FaSSIF pH 6.5			
Sodium taurocholate			3 mM
Lecithin			0.75 mM
NaH ₂ PO ₄			3.438 g
NaCl			6.186 g
NaOH	qs	ad	pH 6.5
Deionized water	qs	ad	1 L

Abbreviation: FaSSIF, fasted-state simulating intestinal fluid.

and the volume of intestinal contents. In response to these needs, attempts were made to create a biorelevant medium based on experimental data from the literature (6,7). Specifically, fasted-state simulating intestinal fluid (FaSSIF), containing physiologically relevant concentrations of bile salts and phospholipids (lecithin) and having a pH that is representative of values measured from the mid-duodenum to the proximal ileum and a buffer capacity that is comparable with typical fasted-state values measured from fasted human intestinal juice, was developed to simulate fasting conditions in the proximal small intestine. The composition of FaSSIF is given in Table 2.

Media to Simulate the Upper GI Tract in the Fed State

In the fed state, the luminal composition in the stomach will be highly dependent on the composition of the meal ingested. Simple aqueous buffer media are not at all suitable to simulate such conditions since they fall short of a realistic simulation of postprandial gastric and SI conditions.

Milk and complete nutrition products (Ensure[®] Plus). Milk has been investigated for use as a dissolution medium (15,16). Typically, standardized, homogenized cow's milk with a fat content of 3.5% (whole milk) is used. Milk has a similar composition to a standard breakfast (17) with respect to the ratio of carbohydrate to fat to protein. However, milk has also some shortcomings in terms of pH and simulating gastric secretion and digestion. Further to avoid stability problems, heat-treated milk should be used.

With the intention of simulating gastric conditions after a Food and Drug Administration (FDA) standard breakfast, Ensure Plus, a complete nutritional fluid was proposed as a dissolution medium (18). Ensure Plus is a good alternative to milk when it is necessary to closely resemble initial gastric conditions after administration of a high-fat meal. However, as for milk, it has to be considered that with Ensure Plus alone, it is not possible to simulate the changes in gastric secretion and digestion with time. Thus, for MR formulations sensitive to the latter factors, it is necessary to additionally simulate these processes.

Fed-state simulating intestinal fluid. Conditions for drug dissolution in the proximal part of the small intestine are highly dependent on whether the drug is dosed in the fed or fasted state. After ingesting a meal the pH of the chyme is lower than the intestinal fluid pH in the fasted state, while buffer capacity and osmolality show a sharp increase. Along with these factors, the sharp increase in bile output could also be a major influence on the bioavailability. Furthermore,

TABLE 3 Composition of the Biorelevant Medium Used to Simulate Fed-State Conditions in the Small Intestine

FeSSIF pH 5.0			
Sodium taurocholate			15 mM
Lecithin			3.75 mM
Acetic acid			8.65 g
NaCl			11.874 g
NaOH pellets			4.04 g
Deionized water	qs	ad	1 L

Abbreviation: FeSSIF, fed-state simulated intestinal fluid.

specific interactions between the drug and ingested food components may occur. A dissolution medium for simulating the fed-state small intestine should reflect all of these factors. Fed-state simulated intestinal fluid (FeSSIF), a medium with a high buffer capacity and osmolarity, a pH value representative of fed-state conditions in the small intestine and bile components that are present in considerably higher concentrations than in the fasted-state medium at least partially meets these requirements. The composition of the FeSSIF is given in Table 3.

Media to Simulate Conditions in the Proximal Colon

When a dosage form passes through the ileocecal junction and enters the cecum, it is confronted with a different intraluminal environment. In contrast to the gastric and SI environment, large numbers of bacteria are present in the cecum. These bacteria exhibit a high metabolic activity, resulting in extensive fermentation. Colonic bacteria species are able to digest a number of food products that are not digested by pancreatic enzymes, such as some of the complex sugars contained in dietary fiber and fatty acid esters. A major product of the bacterial hydrolysis of carbohydrates (CHOs) are short-chain fatty acids (SCFAs) like butyrate, propionate, and acetate. The production of SCFAs results in a decreased pH value in the proximal colon. Intrasubject and intersubject variations in colonic pH are large. Typical pH values that have been measured in the proximal colon range from 5.5 to 6.8 (19–22). With transit along the colon, the SCFAs are absorbed or neutralized by bicarbonates. Hence, intraluminal pH rises again to neutral values in the descending colon. To simulate the composition and the physicochemical characteristics of the contents of the proximal colon, simulated colonic fluid (SCoF) has been developed (23). This medium has a slightly acidic pH and contains acetate ions to represent at least one of the typical ions that can be found in this segment of the GI tract. The composition of SCoF is shown in Table 4.

TABLE 4 Composition of the Biorelevant Medium Used to Simulate Conditions in the Proximal Colon

SCoF pH 5.8			
1 M Acetic acid			170 mL
1 M NaOH			157 mL
Deionized water	qs	ad	1 L

Abbreviation: SCoF, simulated colonic fluid.

Instrumental Parameters for USP Apparatus 3

Various experiments have been performed to study the impact on instrument/test parameters, particularly media volume, agitation rate, and mesh screen sizes, on drug release from a selection of formulations representing different types of release mechanisms. Results suggest that media volumes of about 190 to 220 mL are adequate. When using smaller volumes (≤ 180 mL) of media, the glass cylinders cannot be completely filled during a downstroke motion. But when using higher volumes (≥ 230 mL), vessels can spill over when the glass cylinder starts its downstroke. Hence, volumes of 200 to 220 mL are assumed to be optimal (24).

Drug release from many MR formulations, particularly that from erosion-based delivery systems, is affected by the hydrodynamic conditions in the GI tract. Therefore, to predict *in vivo* drug release, it would be highly desirable to mimic these conditions in the *in vitro* setup. However, as GI passage is a highly variable dynamic process that is characterized by intermittent phases of agitation and quiescence (25), its simulation is quite a challenge. Therefore, in the *in vitro* approach, it is necessary to identify average agitational rates as a compromise. For this purpose, agitational rates in the range of 10 to 20 dpm proved to be adequate (24,26).

Tests performed with different combinations of mesh screens at top and bottom of the glass cylinder indicated that when using 74 μm mesh screens as either bottom or top mesh, the glass cylinders fail to drain (24,26,27). This prevents an adequate exchange between the medium in the inner and the outer tube of the setup and most likely results in artefactual hydrodynamic conditions during the test. Similar observations can be made with 150 μm mesh screens as the top mesh, particularly with dip rates ≥ 10 dpm, that are typical reciprocating rate used in BioDis experiments. In contrast, the use of 420 μm screens at bottom and top of the glass cylinders results in complete and rapid drainage (24,26,27). Therefore, the use of bottom meshes of ≥ 150 μm and top meshes ≥ 420 μm is highly recommended when the aim of the setup is to obtain "standardized" and reliable test conditions.

Predicting Drug Release from Delivery Systems Intended for Site-Specific Release

Most orally administered solid dosage forms are intended to deliver the drug systemically. Typically the drug is released from the dosage form and then absorbed in the small intestine, after which it appears in the systemic circulation and is conveyed to the site of action. However, in recent years there has been a significant increase in available strategies for site-specific delivery in the GI tract both to maximize a therapeutic response and to reduce side effects. Although the number of site-specific delivery systems is increasing, in only a few instances has attention been paid to how these products will perform in the heterogeneous environment of the human gut.

Site-specific delivery systems can bring great benefit for various drugs, for example, mesalazine, an anti-inflammatory drug used to induce and maintain remission of inflammatory bowel disease (IBD) such as Crohn's disease (CD) and ulcerative colitis (UC). Mesalazine is intended to act locally at the inflamed sites of the GI tract. Therefore, the delivery objective for oral treatment with this drug is to achieve high concentrations of the active moiety at the sites of

inflammation while minimizing systemic absorption. Release of drug in the proximal GI tract (stomach and upper small intestine) should be avoided to circumvent premature absorption and consequent “drug wastage” and systemic side effects. Currently marketed formulation concepts for oral mesalazine treatment of IBD include (i) tablets coated with enteric polymers, (ii) microspheres (multiparticulates) that release the active drug via diffusion-controlled mechanism, and (iii) enteric-coated microspheres that are intended to release the active compound in a pre-determined rate after the coating has dissolved in the small intestine.

To evaluate the ability of different site-specific drug delivery systems containing mesalazine to release drug at various locations within the GI tract, and thus, to identify which formulations are suitable for various subgroups within CD and UC patients, it is necessary to employ a dissolution method that is able to reflect the changing environment as a dosage form housing the anti-inflammatory agent moves through the GI tract. Experiments with single media in a simple paddle setup, as shown in Figures 4 and 5, are not sufficient for this purpose.

Results from the paddle experiments indicate that the multiparticulate formulations release the drug in a controlled manner over time, whereas the onset of drug release from the tablet formulations is strongly dependent on the pH of the test medium. In simulated intestinal fluid *sine pepsin* (SIFsp) pH 6.8, a medium reflecting pH conditions in the mid-jejunum, pronounced differences in the lag times before the onset of drug release are obvious in the profiles (Fig. 4). In contrast, even for formulations with different types of enteric coatings (Fig. 5), these differences are eliminated when the pH of the medium is adapted to pH conditions corresponding to the terminal ileum (SIFsp pH 7.5). These results show that experiments in single media can be useful to illustrate the differences in drug-release mechanisms of the mesalazine formulations, but that they

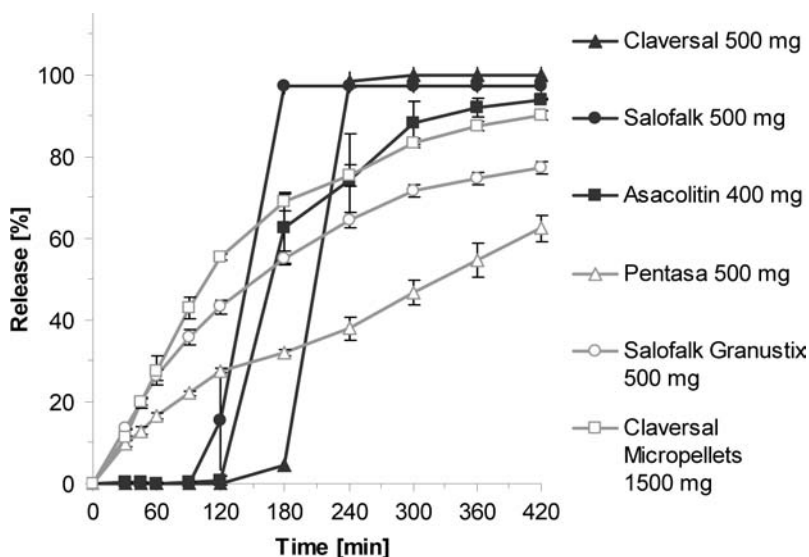


FIGURE 4 Dissolution behavior of monolithic (closed symbols) and multiparticulate (open symbols) mesalazine dosage forms in SIFsp pH 6.8, USP apparatus 3.

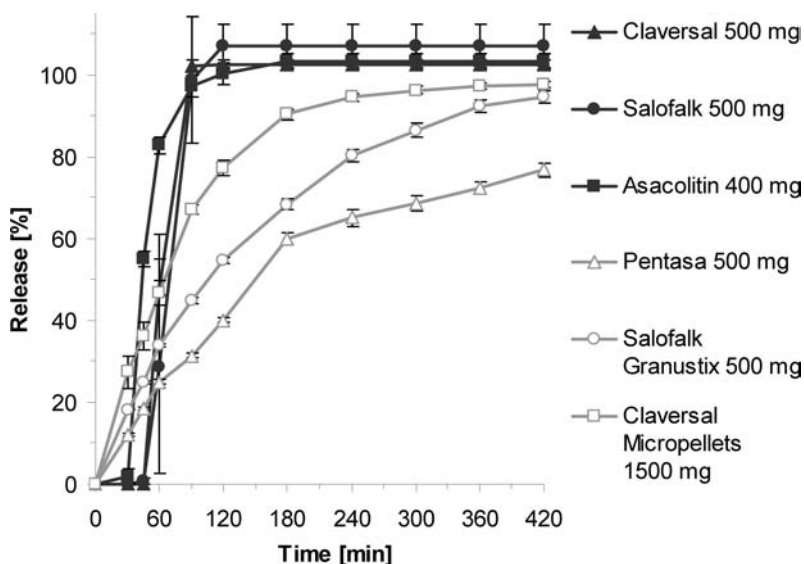


FIGURE 5 Dissolution behavior of monolithic (*closed symbols*) and multiparticulate (*open symbols*) mesalazine dosage forms in SIFsp pH 7.5, USP apparatus 3.

cannot be used to differentiate between the dosage forms in a way that could easily be interpreted in terms of relative ability to deliver mesalazine in a targeted manner to inflamed regions of the gut. In contrast, the BioDis equipped with a gradient of buffers with physiological pH values or biorelevant media is entirely appropriate for this purpose. Combined with physiologically based residence times in the respective media, such a pH gradient results in test conditions that are convenient and discriminating for comparing the drug-release behavior from dosage forms of mesalazine and other drugs that need to be delivered to specific sites in the GI tract. Results from biorelevant pH-gradient studies might therefore be very helpful in terms of deciding which dosage form should be administered to the patient to optimally address the localization of the inflamed areas. Since most of the mesalazine dosage forms are enteric-coated formulations, they have to be administered to the patient in the fasted state. Thus, the pH gradient used to screen these formulations should be composed to reflect fasted GI conditions. Table 5 illustrates the pH values, media, and the corresponding residence times that can be used to simulate a passage through the fasted human GI tract for such products.

Figure 6 shows the results from the biorelevant setup. It is obvious that the pH and the residence time in the different segments of the GI tract are the main determinants of the drug release from the site-specific delivery systems of mesalazine and that based on the size and the composition of the formulations, drug release will occur at different sites in the GI tract.

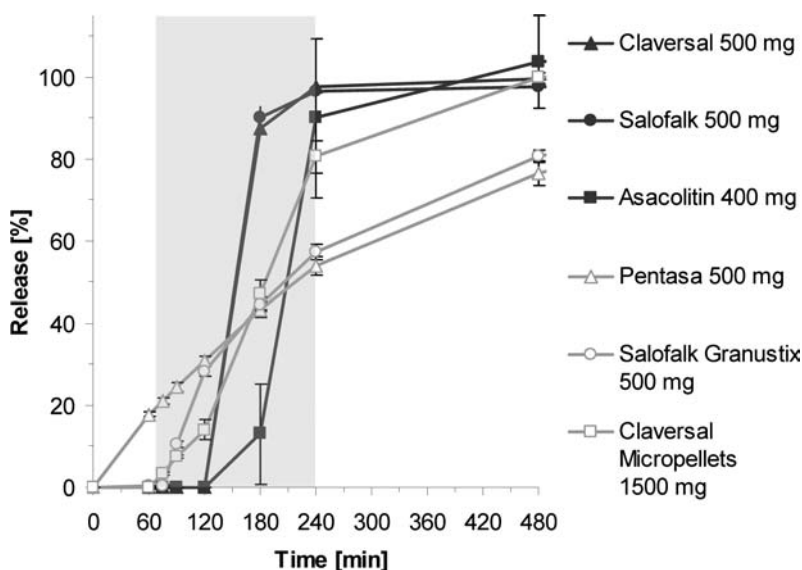
None of the enteric-coated dosage forms released any drug under gastric conditions within the test duration. Assuming human GI pH profiles and passage times similar to those used in the present study, the tablet formulations Salofalk[®] and Claversal[®] are likely to release nearly the whole amount in the proximal ileum and therefore are likely to be most effective if the main site of

TABLE 5 Dissolution Media and Transit Times Reflecting a Passage Through the Fasted Human GI Tract

GI segment	pH	Compendial medium	Biorelevant medium	Transit time	
				Tablets (min)	Pellets (min)
Stomach	1.8	SGF ^a	SGF plus	60	60
Proximal jejunum	6.5	Phosphate buffer	FaSSIF	15	45
Distal Jejunum	6.8	SIFsp	FaSSIF ^{a,b}	15	45
Proximal ileum	7.2	Phosphate buffer	FaSSIF ^{a,b}	30	45
Distal ileum	7.5	SIFsp USP 23	Blank FaSSIF ^a	120	45
Proximal colon	5.8	Acetate buffer	SCoF	360	360
Proximal colon	5.8	Acetate buffer	SCoF	240	240
Distal colon	6.8	SIFsp	Blank FaSSIF ^a	360	360
Distal colon	6.8	SIFsp	Blank FaSSIF ^a	240	270

^apH modified.^bConcentration of bile components modified.

Abbreviations: GI, gastrointestinal; SGF, simulated gastric fluid; FaSSIF, fasted-state simulating intestinal fluid; SIF, simulated intestinal fluid; SCoF, simulated colonic fluid.

**FIGURE 6** Dissolution behavior of single-unit (*closed symbols*) and multiple-unit (*open symbols*) mesalazine dosage forms in a physiological-based pH gradient (*shaded area* represents residence time in the small intestine) method, USP apparatus 3.

inflammation is found in the ileum. The onset of drug release from the Asacolitin[®] tablet will most probably take place in the more distal ileum, which can result in benefit for patients who suffer from inflammation primarily in the terminal ileum and proximal colon. By contrast, in patients where only the colon is inflamed, nearly the whole amount of drug will be released from all tablet formulations well before reaching the inflamed areas. A significant amount of

drug will therefore be prematurely absorbed in the small intestine, resulting in an increased risk of side effects and inadequate concentrations of drug substance at the inflamed areas in the colon (28).

On the basis of their release profiles, all multiparticulate formulations are intended for the treatment of inflammation that spreads throughout the whole small intestine and proximal colon. Since the Pentasa[®] formulation starts to release the active drug as early as in the stomach, this formulation is particularly appropriate for those patients who suffer from gastric inflammation. However, in the majority of patients a substantial drug release in the stomach would represent drug wastage (loss of active drug due to systemic absorption) combined with an increased risk of adverse effects.

Overall, from the present study it is obvious that the selection of the dosage form to be administered can strongly influence the outcome in an individual patient. It is also clear that none of the described mesalazine dosage forms represents an optimal drug delivery system for colonic delivery and that there is definitely a need for dosage forms that can deliver drugs to the colon in a more specific way.

Case Study: Predicting the In Vivo Release Behavior of a Novel pH- and Time-Based Multiunit Colonic Delivery System

Because of the need for better therapy of the diseased colon, much interest has been focused in recent years on site-specific delivery to the colon. A few years ago, a novel type of delivery system has been developed for the treatment of UC, representing a combined pH- and time-based multiunit dosage form (29) to localize release of mesalazine insofar as possible to the afflicted sites in the colon. The system consists of a mesalazine core, which is coated first with a blend of two pH-independent polymers to produce a slow release of mesalazine from the pellets, and secondly, with an enteric polymer that dissolves rapidly at $\text{pH} \geq 7.2$ and was used to delay the onset of drug release until the pellets reached the terminal ileum.

To evaluate the in vivo performance of this novel formulation, a proof of principle study was to be conducted. To monitor the rate of release from the prototype during the GI passage and subsequent absorption via plasma sampling, a prototype containing caffeine, a marker drug being rapidly and completely absorbed along the entire GI tract, was used. To check for the predictive power of the pH-gradient method in terms of site and timing of drug release of the prototype before starting the in vivo study, the drug-release profile of the prototype was examined with the fasted pH-gradient method.

Dissolution and IVIVC. In simulated fasted-state conditions, the prototype was shown to start releasing the active drug in the ileum followed by a controlled release along the colon, meeting the goals of the formulation project.

After obtaining the in vivo data from 12 healthy volunteers, the absorption kinetics of caffeine were estimated by the Wagner-Nelson method. Serum concentrations were used to determine various pharmacokinetic parameters. Subsequently, the fraction of dose absorbed (f_{abs}) was calculated by using the mean plasma concentration-time profile.

To better compare the dissolution profile generated with the pH-gradient method with that representing the fraction absorbed at corresponding time

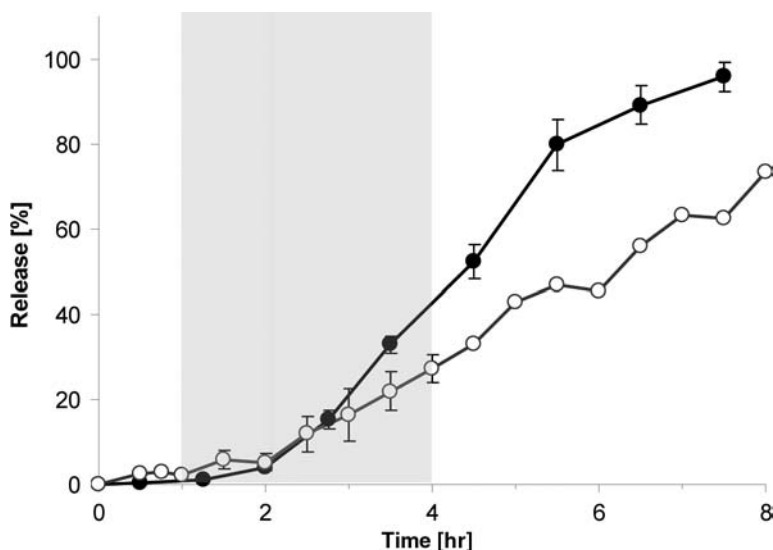


FIGURE 7 Comparison of the mean fraction absorbed in vivo (○) and the mean fraction released in vitro (●) over the same time range (shaded area represents residence time in the small intestine) using USP apparatus 3 and biorelevant conditions (Table 5).

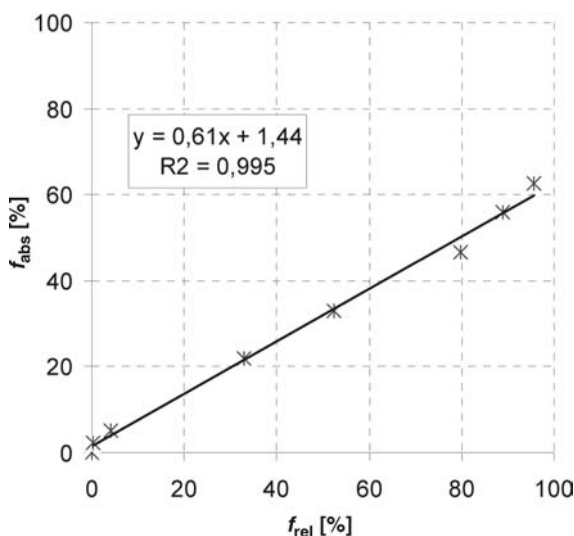


FIGURE 8 Relationship between the mean fraction absorbed (f_{abs}) in vivo and the mean fraction released (f_{rel}) in vitro. The line represents the linear regression of the data where $f_{abs} = 0.61f_{rel} + 1.44$ and $R^2 = 0.995$.

points, the profiles were compared plotting the calculated values over the same time range (Fig. 7).

To further elucidate the predictive power of the in vitro test setup, the f_{abs} calculated from the mean plasma concentration–time profiles at distinct time points was also plotted versus the fraction released (f_{rel}) in vitro using a Levy plot (Fig. 8).

The Levy plot shown in Figure 8 indicates a good correlation between the *in vivo* fraction absorbed and the *in vitro* drug release and demonstrates that the pH gradient is useful in terms of predicting the timing/site of drug release from the colonic delivery system (30). The slope of the plot of percentage of drug released against the percentage absorbed was less than one. This can likely be attributed to a slower absorption process, which of course is not simulated in the *in vitro* release experiments. Previous authors working on IVIVC have also observed that, in general, *in vitro* results tend to run ahead of *in vivo* data.

Overall, a good IVIVC was obtained and from both *in vitro* and *in vivo* studies, it can be concluded that the novel pH- and time-controlled multiunit delivery system would dramatically improve selectivity of drug delivery to the distal ileum and the colon and therefore could be beneficial in both UC and other colon-related diseases (30).

Results from the present case study indicate that the biorelevant methodology offers an excellent tool that can be used in development of new formulations. Particularly for the treatment of IBD patients, a patient-specific treatment based on a clear diagnosis regarding type, localization, severity, and extent of the inflammation in CD or UC in combination with the established biorelevant release profiles should be invoked to optimize the therapy.

Predicting Drug Release from Extended-Release Oral Dosage Forms

The examples in the preceding sections illustrate the utility of USP apparatus 3 and the use of a pH gradient to simulate drug release from site-specific delivery systems, particularly enteric-coated formulations that are administered to the patient in the fasted state. However, drug release from these and other kinds of MR dosage forms should be robust regardless of when the dosage form is given in relation to meal intake. Therefore, it would be of great benefit to develop an *in vitro* test method that can discriminate dissolution performance among extended-release (ER) dosage forms of a given drug, with view to predicting *in vivo* differences after fasted- and fed-state administration. ER formulations containing theophylline, an antiasthmatic drug, are a good case example for this purpose. Theophylline belongs to the narrow therapeutic index drugs. As is typical for these activities, the efficacy and toxicity of theophylline are highly dependent on its plasma concentration. Thus, it is very important to maintain serum drug levels in the therapeutic range. For this purpose, theophylline doses should be adjusted for each individual patient by therapeutic monitoring. As the elimination half-life of theophylline is short (4–9 hours), ER dosage forms are the formulations most favored for the long-term management of chronic asthma. An ideal ER product should demonstrate complete bioavailability, minimal fluctuations in drug concentration at steady state, reproducibility of release characteristics independent of food, and minimal diurnal variation. However, with the first ER formulations, it became clear that not all meet the requirements of an ideal theophylline ER product. It was shown that in many cases drug release from various theophylline ER formulations could be influenced (either increased or decreased) by concomitant intake of food. Although in maintenance therapy of asthma most drugs are given in conjunction with food, the recent literature however contains very few *in vivo* studies and next to no *in vitro* investigations of the influence of food on the bioavailability of theophylline and other drugs

from ER formulations. However, food intake can influence the rate of drug release from the dosage form, the rate of drug absorption or the amount of drug absorbed, or all of these parameters simultaneously. This, in turn, can result in an unexpected shift of the plasma theophylline concentration. In particular, sudden release of the entire ER dose (dose dumping) can and does result in toxic plasma concentrations (31).

In the USP, various dissolution methods are described for examining drug release from theophylline ER products. However, in terms of predicting the *in vivo* release behavior, the compendial methods are not capable of simulating the critical physiological conditions, neither with respect to pH values and passage times through different sections of the GI tract, nor with respect to the presence of food and/or bile components. The BioDis, equipped with sets of media reflecting the fasted- and fed-state environment along the GI lumen, seems more appropriate to predict *in vivo* behavior of different theophylline ER dosage forms under different dosing conditions.

Case Study: Predicting Food Effects on Drug Release from Theophylline ER Formulations

To examine whether it is possible to detect the influence of food on drug release of different types of ER formulations, various marketed theophylline ER formulations including coated multiparticulates and monolithic matrix formulations were screened with biorelevant pH-gradient methods simulating fasted- and fed-state dosing conditions. Analogous to the previous studies, the GI passage through the upper GI tract was first simulated using a compendial pH gradient and then a corresponding test was performed using biorelevant media to simulate further parameters that may be crucial for *in vivo* drug release. To achieve the main objective of the studies, that is, to check whether drug release from the different dosage forms is influenced by fasted- versus fed-state dosing conditions, a new gradient method was designed to simulate passage through the fed-state GI tract after (i) a standardized high-fat breakfast and (ii) a light breakfast. Not only the different intragastric and intrainestinal conditions but also the longer gastric residence times of nondisintegrating dosage forms that are typically observed after fed-state administration were accounted for in the fed-state dissolution model. Table 6 illustrates the test conditions that were used to simulate the fed-state GI passage with compendial and biorelevant media.

The resulting drug-release profiles indicate that the theophylline ER formulations vary in their sensitivity to different dosing conditions. Comparing release profiles generated with the fasted- and fed-state compendial gradient, it was obvious that none of the dosage forms exhibits pH-dependent drug release in the GI pH range. Moreover, the resulting profiles from the two compendial pH gradients were nearly superimposable for all dosage forms tested. Whereas, for example, drug release from ethylcellulose-coated multiparticulate formulations proved not to be dependent on the composition of the media and the corresponding residence times, for some formulations tested, there were considerable differences in drug release under simulated preprandial versus postprandial dosing conditions. This was particularly the case for the tablet formulations (Figs. 9 and 10).

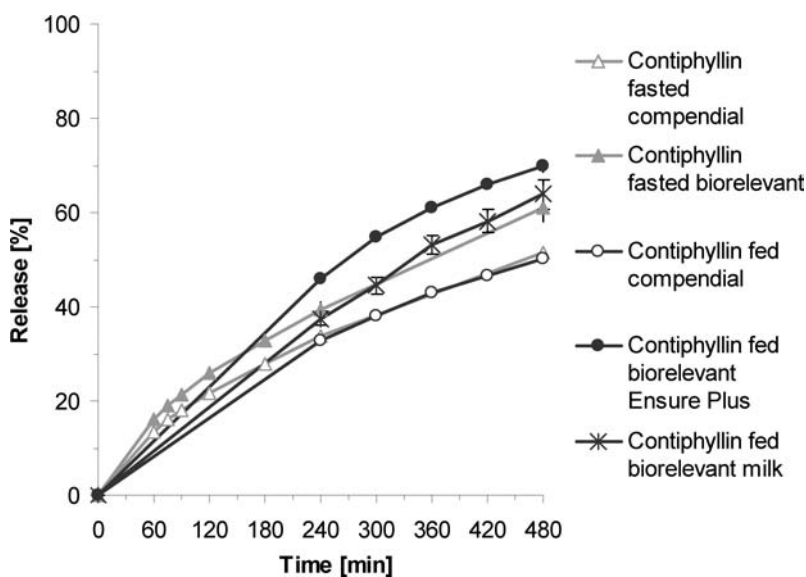
Concentrations of bile components corresponding to those of the fasted intestinal lumen led to merely a slight increase in drug release from both tablet

TABLE 6 Dissolution Media and Transit Times Reflecting a Passage Through the Fed Human GI Tract

GI segment	pH	"Compendial" medium	Biorelevant medium	Transit time	
				Tablets (min)	Pellets (min)
Stomach	5.0	Blank FeSSIF	a) Ensure [®] Plus, b) Milk	240	120
Proximal jejunum	5.0	Blank FeSSIF	FeSSIF	15	45
Distal jejunum	6.5	Blank FaSSIF	FeSSIF ^{a,c}	15	45
Proximal ileum	6.8	Blank FaSSIF ^a	FeSSIF ^{a,b,c}	30	45
Distal ileum	7.5	Blank FaSSIF ^a	Blank FaSSIF ^a	120	45
Proximal colon	5.8	Acetate buffer	SCoF	360	360
Proximal colon	5.8	Acetate buffer	SCoF	240	240

^apH modified.^bConcentration of bile components modified.^cPhosphate buffer.

Abbreviations: GI, gastrointestinal; FeSSIF, fed-state simulated intestinal fluid; FaSSIF, fasted-state simulating intestinal fluid; SCoF, simulated colonic fluid.

**FIGURE 9** Dissolution profiles of Contiphyllin[®] 300 mg tablets under fasted- and fed-state conditions.

formulations. Increasing the concentration of bile components to those typical of the fed state, drug release further increased. For Contiphyllin[®] tablets, the increase was relatively modest. In the case of Tromphyllin[®] retard tablets, however, results generated with the biorelevant gradients indicate an increased release rate when the tablet is taken with or after a high-fat meal (i.e., the FDA high-fat standard breakfast). This would be associated with a pronounced increase in the rate of absorption, placing the patient at a greater risk of toxicity.

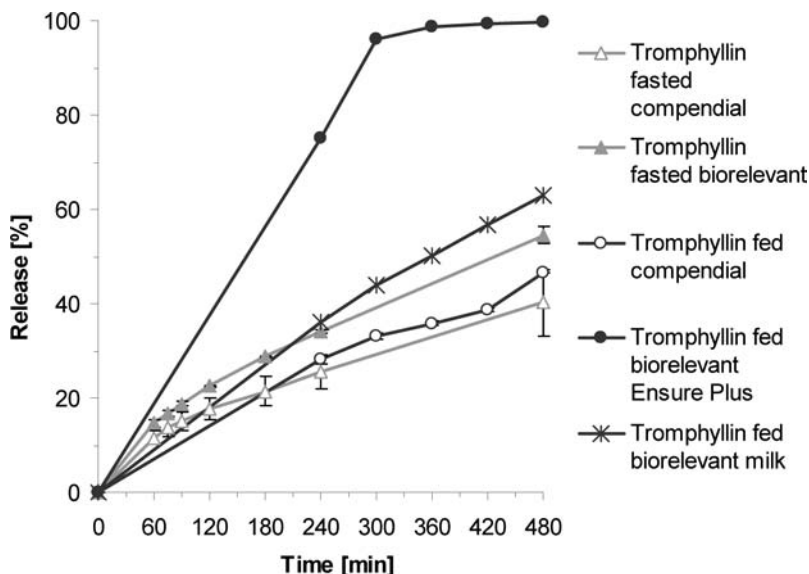


FIGURE 10 Dissolution profiles of Tromphyllin[®] retard 300 mg tablets under fasted- and fed-state conditions.

However, when simulating the postprandial stomach with milk to mimic administration with a light breakfast, drug release from Tromphyllin retard tablets was not markedly affected.

To better characterize the influence of a high-fat meal on drug-release rate in the postprandial stomach, a further set of experiments was performed. The main objective of this series of tests was to check what might be the reason for this increased release rate and whether the release of almost 80% of the active drug during gastric residence occurred via dose dumping or if drug release occurred at a steady state over the course of gastric residence. Drug-release profiles of Contiphyllin and Tromphyllin retard tablets generated under postprandial gastric conditions are summarized in Figure 11.

Dissolution profiles clearly indicate that food effects on drug release from Tromphyllin did not result in a bolus dose dumping but, compared to Contiphyllin, there was a much higher, albeit zero-order, drug-release rate. This observation was in good agreement with the appearance of the tablets when they were inspected after their residence in gastric medium. As expected for a diffusion-controlled drug release, Contiphyllin tablets were swollen but still intact, irrespective of the test medium used. Using compendial media or milk, the same was observed for Tromphyllin tablets. By contrast, in Ensure Plus, approximately half of the original matrix from Tromphyllin tablets was lost by erosion within the same time frame, with correspondingly high drug release. In this case, release was controlled by both diffusion and erosion. The erosion, that is, the weak integrity of the gel layer of Tromphyllin, might derive from various factors, for example, the drug/HPMC ratio, the viscosity of HPMC, and the type and amount of further excipients. A further explanation might be the osmotic pressure generated by various electrolytes in the postprandial gastric medium

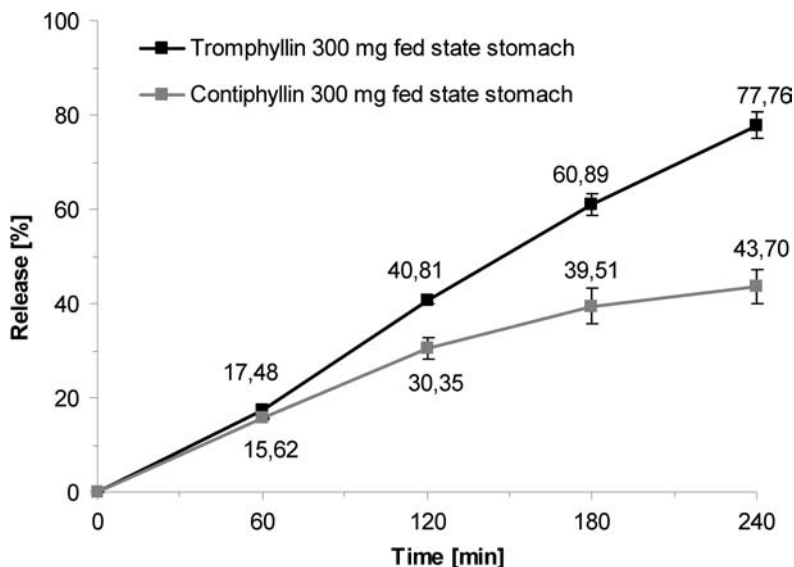


FIGURE 11 Drug-release rates of Contiphyllin[®] 300 mg and Tromphyllin[®] retard 300 mg tablets under fed-state gastric conditions.

that can contribute to a loss of the integrity of the HPMC gel layer and therefore enhance the erosion process (24).

Overall, these results indicate that drug release from the Contiphyllin tablet is robust under various dosing conditions whereas the drug release from the apparently similar Tromphyllin tablet could be altered by concomitant food intake. These observations are in good agreement with information given in the package insert, according to which a higher maximum plasma concentration in the steady state ($C_{\max/ss}$) was reported when Tromphyllin was administered together with food ($C_{\max/ss}$ fasted $4.9 \pm 1.7 \mu\text{g/mL}$ vs. $C_{\max/ss}$ fed $5.9 \pm 1.7 \mu\text{g/mL}$).

Although there is no direct comparison of pharmacokinetics of the two HPMC formulations available in the literature, it is reasonable to assume that administration immediately after a high-fat breakfast would result in markedly different plasma levels, whereas both tablets should generate very similar plasma levels when given in the fasted state or with a light breakfast. In terms of predicting the in vivo behavior of ER dosage forms, the results from the present series of tests clearly illustrate the importance of choosing suitable in vitro test conditions. The importance of simulating GI conditions with respect to composition and transit in both fasted and fed state when testing extended-release dosage forms cannot be overemphasized.

Summary

For many years the paddle and the basket apparatus and simple aqueous buffers were used to examine the in vitro performance of MR formulations. While these apparatus are useful for quality control purposes, they are not as appropriate in predicting the in vivo performance of these formulations as apparatus 3 and 4. Data presented in this chapter demonstrate that USP apparatus 3 offers the

possibility to closely resemble the GI passage of different types of MR dosage forms and can also be used to simulate different dosing conditions. Overall, this methodology can be applied to screen MR formulations throughout the development chain and can be used to indicate dosage form derived risks and benefits for the patient. Thus, it offers many benefits for both the formulator and the patient. As the biorelevant pH gradients can also be adapted to simulate pH profiles and passage times in specific patient subgroups, their application offers various opportunities for making better formulations in the future.

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Modified-Release Dosage Forms: Formulation Screening in the Pharmaceutical Industry

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INTRODUCTION

Oral modified-release (MR) formulation is designed to deliver the drug to the body at a predetermined rate or site in the gastrointestinal (GI) tract. Oral MR includes, for example, extended-, controlled-, prolonged-, sustained-, delayed-, and pulsatile-release formulations. The focus of this chapter is formulations providing a slower drug release compared with conventional immediate-release (IR) formulations and the term extended release (ER) will be used to refer to these formulations.

MR formulations in the form we know them have been available for more than half a century. The interest in the area has been constantly growing, which is exemplified by the number of scientific publications and patents (Fig. 1). Furthermore, of the 50 best selling drugs in the United States, 20% were oral MR formulations and within AstraZeneca, a major pharmaceutical company, about one fourth of the new chemical entities (NCEs) presently in late clinical development have been developed from the outset as MR formulations. Thus, oral MR formulations maintain an important position, and if anything are still growing, within the area of pharmaceutical product development.

A drug product is more than a molecule, which is nicely illustrated by MR formulations since they can significantly improve the therapeutic efficacy, tolerability and patient convenience. Initially, MR formulations were mainly regarded as a way to improve patient compliance by allowing simplified dosing schedules, for example, once-daily dosing for drugs which otherwise would have needed more frequent intake. The MR products were then introduced to the market as line extensions following the first launch of an NCE as a conventional IR formulation. However, during recent years MR formulations have been viewed more and more as a way to optimize clinical properties of an NCE. The basic concept for ER formulations is to maintain the plasma concentrations within the therapeutic interval thereby avoiding undesired effects related to peak plasma concentrations and subtherapeutic trough levels (Fig. 2). Another formulation type which has been used for a long time is the enteric coated formulation, which prevents acidic drug degradation or local irritation in the stomach, for example, as applied for proton pump inhibitors and anti-inflammatory drugs. Examples of additional

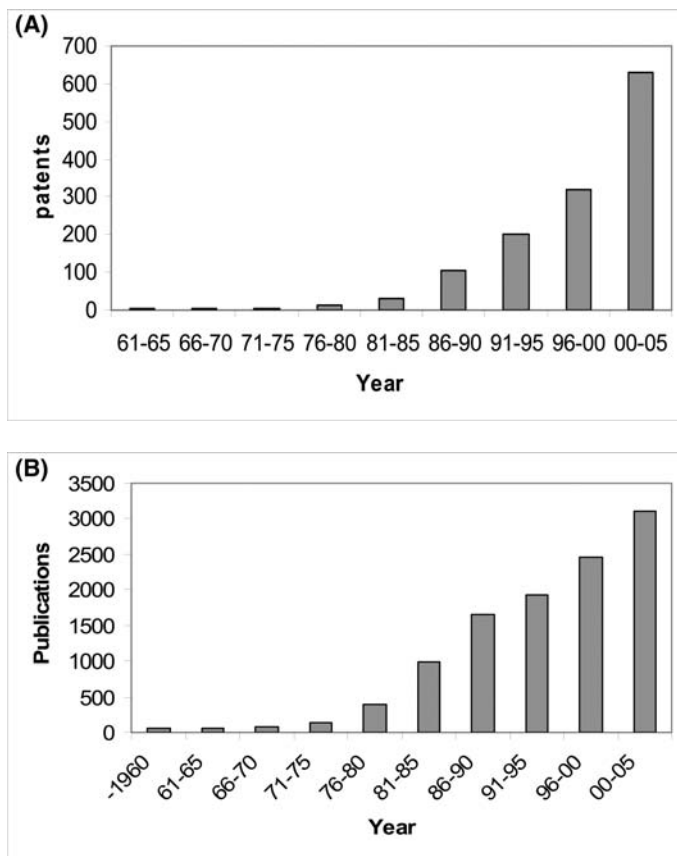


FIGURE 1 The number of **(A)** patents and **(B)** publications in modified-release area (including extended, controlled, sustained, prolonged, and delayed release) until 2005.

mechanisms for improving clinical effect and tolerability by MR formulations include the following:

1. Improving the apparent potency without increasing system exposure. The apparent potency could, for example, be increased by avoiding drug levels, which reach the plateau of the drug plasma concentration and pharmacodynamic effect relationship. This seems to be the case for a long-acting metoprolol ER formulation (1).
2. Improving efficacy by matching diurnal variations of disease factors. For example, an ER formulation of verapamil, an antihypertensive drug, has been developed to provide peak plasma levels in the early morning when the risk of cardiovascular events is at its highest (2).
3. Reducing the extent of drug-drug interaction or drug-food interaction. For example, the increase of felodipine bioavailability induced by grapefruit juice via effects on first-pass metabolism is significantly lower for an ER tablet compared with an IR formulation (3).

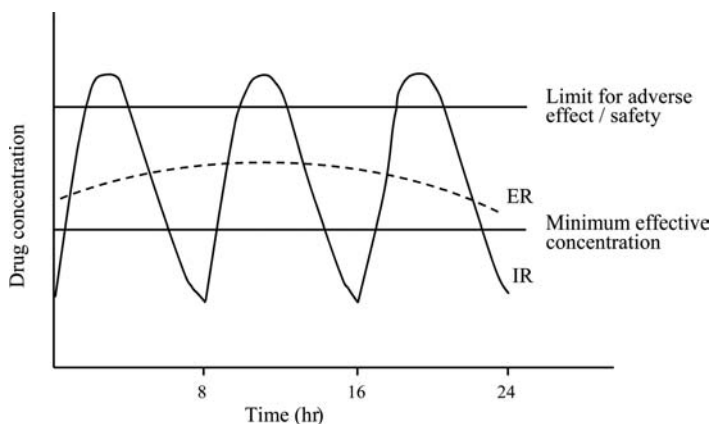


FIGURE 2 Plasma concentration versus time profiles for typical ER and IR formulations, together with indication of desired therapeutic range, illustrating the basic principle of plasma concentration control of ER formulations compared with IR ones. *Abbreviations:* ER, extended-release; IR, immediate-release.

4. Target delivery to a certain area of the GI tract to improve bioavailability by avoiding region specific luminal or gut wall metabolism (see more detail in section “Biopharmaceutical Preformulation: Assessment of Regional Drug Absorption”).
5. Target delivery to a certain area of the GI tract for local treatment or where pharmacological action is triggered through a receptor in the gut. This concept is well established in the area of inflammatory bowel disease (4).

The development of MR products today as “first product to market” of NCEs increases the requirements for a rational development. For example, it is a strong drive in the industry to minimize the time required to develop products to the point where they are ready for a NDA application. Thus, in vivo performance targets should preferably be achieved the first time without iterations of prototype development and testing. This generates a great need to understand and predict in vivo performance of MR formulations. Such knowledge is not only critical for a rational development process but also leads to high clinical quality of the products. This chapter will provide a review of knowledge and methods used for development of oral MR products including studies/predictions of regional drug absorption as an important prerequisite for ER development, drug dissolution from ER formulations in vitro as well as in vivo studies in preclinical models and in man.

There are five basic MR formulation technologies (Fig. 3) which can be applied to different types of dosage forms such single-unit tablets/capsules or a multitude of smaller units given in a capsule, sachet or embedded in a tablet matrix.

- Diffusion membrane coatings
- Osmotic pumps
- Diffusion matrix units

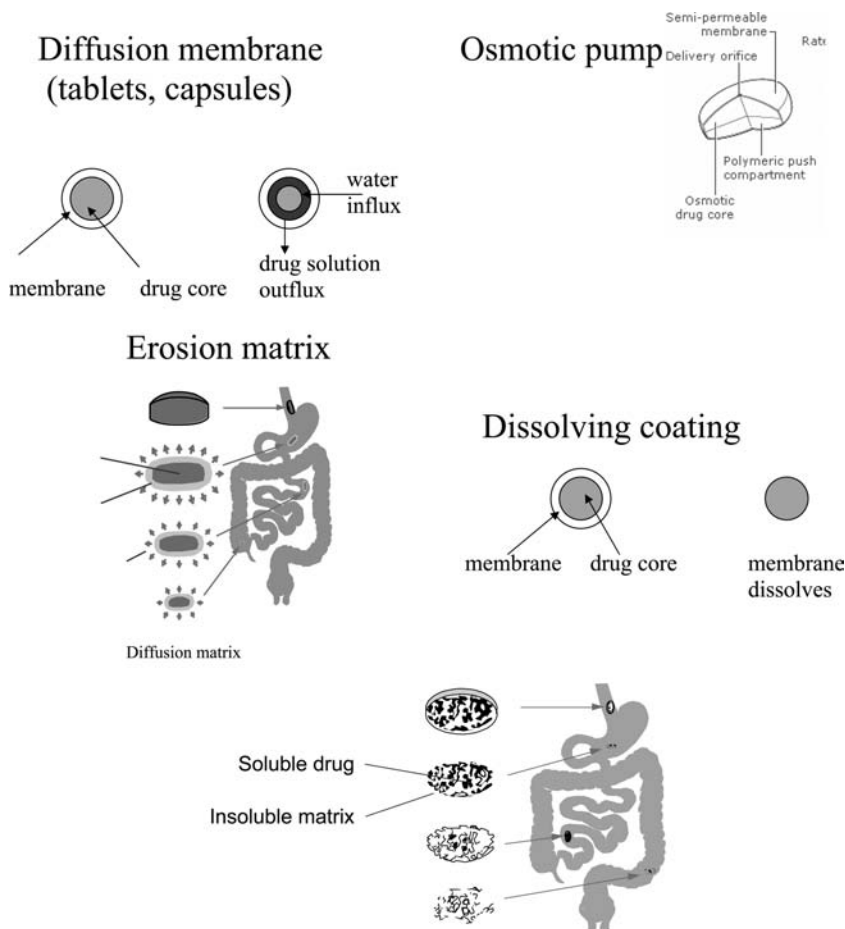


FIGURE 3 Schematic illustration of basic modified-release principles.

- Eroding matrix units
- Dissolving/disintegrating coatings (e.g., enteric coat)

These basic technologies were established more than 30 years ago and almost all products on the market utilize these principles. These technologies provide a versatile toolbox to obtain different release patterns for drugs with different physicochemical properties. Newer systems developed during recent years have often been hybrids combining the basic principles. Typically improvements gained by such combination approaches have at best been incremental. Still, there might be room for further improvements especially in the area of GI targeting. Optimization of formulation performance in the context of physiological and disease factors affecting drug release has been largely neglected and might be another area for additional improvements. Development of a once-daily, robust ER formulation of a high-dose, low-solubility drug would still also be a challenge for most formulators.

COMMON APPROACHES FOR MODIFIED-RELEASE ORAL FORMULATION SCREENING AND EVALUATION

The different steps in development of a MR product are schematically outlined in Figure 4 and described in more detail in the sections below.

Trigger for Development of MR and Target Pharmaceutical Profile

To trigger the development of a MR formulation, there must be information available suggesting that a MR formulation is required and appropriate. For instance, preclinical pharmacokinetic data indicating a short half-life in human is a common trigger for development of an ER formulation. However, plasma concentrations should not be evaluated in isolation, but rather PK/PD relationships for pharmacological effect and/or relationship between plasma drug levels and undesired effects must also be taken into consideration. Drug substance properties such as susceptibility to acid-induced degradation or suspected local irritation of gastric mucosa may require an enteric MR formulation. Furthermore, drugs whose site of action is localized to limited regions of the intestine, for instance specific interactions with intestinal transporters or receptors or for local treatment of inflammatory bowel diseases, may benefit from targeted release formulations. Previous experience from MR formulations of similar drug molecules with similar mechanisms of action is frequently the strongest argument for developing a MR formulation since validated PK/PD models may then be available.

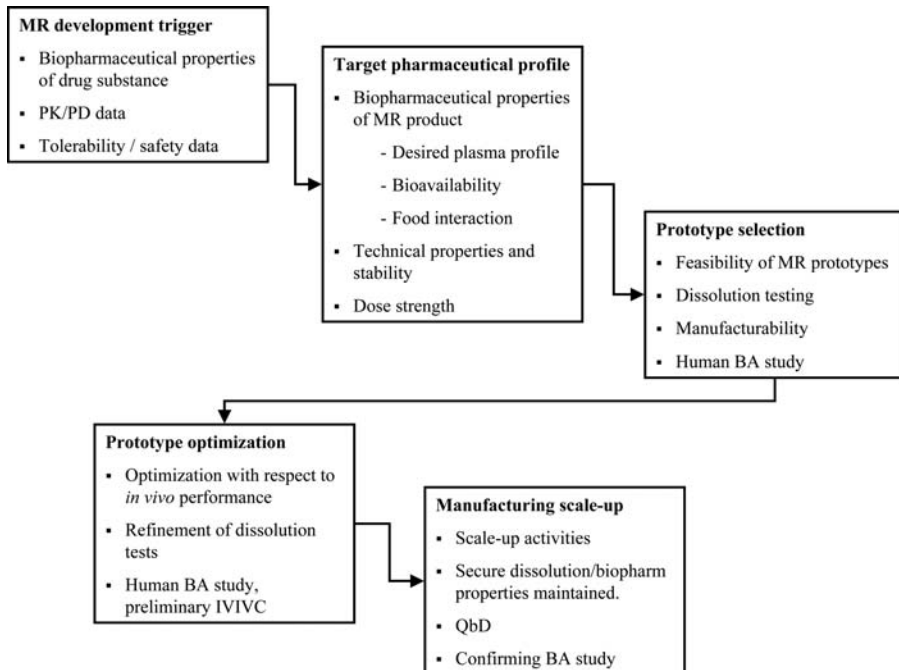


FIGURE 4 Schematic outline of the different steps in the development of a modified-release product.

The first step in a common approach for developing MR oral formulations is to define the target pharmaceutical profile. This would define the desired biopharmaceutical properties of the MR product like peak/trough plasma concentration ratio, relative bioavailability and susceptibility toward variation sources like interactions with food. Furthermore, the expected formulation dose strength is an important aspect to consider in the target pharmaceutical profile. The profile may need to be revised later as clinical data become available, but it is essential to have a target defined to guide the early formulation development work.

Another important input to setting a target pharmaceutical profile is whether the biopharmaceutical drug substance properties demand additional prerequisites or provide additional rationale for MR product development. The solubility is clearly an important property that should be known prior to starting formulation development. The classification of the permeability according to the biopharmaceutical classification system (BCS) is useful also for MR formulations. Low-permeable drugs, that is, class III and IV, may not be suitable for ER formulations because of risk for low bioavailability and high variability. For ER formulations a significant fraction of the dose is released in colon and for that reason it is recommended to assess the permeability in colon. For the same reason, it is desirable to determine the stability of the drug substance both in the small and large intestine. The factors relating to evaluating regional drug absorption will be discussed in further detail below.

Prototype Selection

On the basis of the information in the target pharmaceutical profile, for instance the physicochemical properties of the substance, the expected dose range and the desired in vivo performance of the formulation, a first prototype or set of prototype formulations are developed.

The in vitro screening during this phase reflects the intention to find various suitable prototypes for the drug candidate. The primary aim is to develop compositions and corresponding manufacturing methods for one or several prototype formulations with appropriate in vivo properties. The in vitro screening may consist of excipient and drug substance compatibility testing, stability testing of drug substance and excipients, and functional tests for the formulation including formulation robustness and dissolution testing.

Ideally the formulation development is followed by a human bioavailability study in which a number of prototypes are evaluated. It is recommended to include challenging dosing conditions, for example, concomitant food intake, already in this study. The prototype selection is then based on in vivo performance, also taking into consideration technical aspects of manufacturing, drug and formulation stability, patient convenience, anticipated cost of goods, etc.

Normally the prototype selection phase commences after the initial single ascending dose (SAD) studies, but if existing information and previous knowledge clearly point toward a MR formulation, the prototypes may be evaluated already in the SAD or multiple ascending dose (MAD) studies. In this way considerable development time may be saved.

Prototype Optimization and Preliminary IVIVC

Following the prototype selection, the MR formulation is optimized with respect to in vivo performance including drug release profile, food interactions,

bioavailability, variability, etc. Optimization with respect to the technical properties of the formulation is also common during this phase. Furthermore, it is important to continuously build knowledge about the manufacturing process to facilitate process development and scale-up.

In vitro dissolution is a key tool in optimization of a prototype formulation, and test strategies will be needed, most often comprising multiple tests. Subsequent studies using preclinical in vivo models are not mandatory but could be merited when there is insufficient confidence in in vitro testing. It should be emphasized that during the entire formulation screening, in particular during prototype optimization, knowledge about the formulation and the product attributes that may influence the clinical performance should continuously be accumulated. Scientifically based characterization of the product and the manufacturing process is a vital part of the quality by design approach and much of the foundation for this approach will preferably already be in place prior to scale-up activities.

Similar to the prototype selection phase, the optimization is ideally completed by a human bioavailability study. In the approach described here, the results from this clinical study will be guiding for the selection of formulation for continued clinical studies in patients, for example, dose-finding studies. The study can also be designed to allow for bridging between the MR formulation and the SAD and MAD study formulations, which are often simple solutions and suspensions. Preferably, challenging dosing conditions, such as concomitant intake of food, are included in such a study.

A number of optimized prototype variants may be evaluated in such a bioavailability study. For ER formulations, the variants could consist of formulations with different drug release rates. A great advantage of including formulations with different drug release rates is that the results may form a basis for a preliminary in vitro-in vivo correlation (IVIVC, see also chap. 19). Such a preliminary IVIVC may become very useful in later scale-up and manufacturing process development activities. It can also guide the design of a formal IVIVC study later in the clinical development program.

To better understand the in vivo performance of the MR formulation in vivo imaging is an attractive approach. Such studies are often very informative regarding the formulation function in vivo. In vivo imaging is preferably conducted early during prototype optimization to fully exploit its potential for guiding further development.

Manufacturing Scale-up

When a formulation has been selected on the basis of evaluation of formulations manufactured on a laboratory scale, the manufacturing scale-up and large-scale process development can commence. Knowledge about critical product and manufacturing factors that influence the in vivo performance is increased in this phase. Both the formulation composition and manufacturing process may have to be changed to maintain the product attributes when manufactured on larger scale.

The key biopharmaceutical aspect in this phase is to maintain the clinical properties obtained in prototype optimization. The in vitro testing is largely the same as during the prototype optimization phase, but the purpose is to assure that the dissolution properties are unchanged during scale-up. More emphasis is

also put on the quality by design work and to identify the critical quality attributes of the MR formulation. The impact of changes in the critical quality attributes on dissolution and eventually on the clinical performance should be quantified.

It may be necessary, depending on scientific based risk assessment (including level of changes, likelihood of a difference and clinical impact of potential difference in formulation performance), to conduct a confirmatory bioavailability study with the large-scale formulation to verify that the *in vivo* performance is acceptable. Such a study could have the design of a bioequivalence study.

BIOPHARMACEUTICAL PREFORMULATION: ASSESSMENT OF REGIONAL DRUG ABSORPTION

A dosage form administered under fasting conditions will reach the colon in most instances within three to six hours (5). Thus, if a longer duration of drug release and absorption is desired, which normally is the case, drug absorption in colon is a prerequisite to the successful implementation of an ER strategy. The colon has been questioned as a suitable area for drug absorption. Although shown not to be generally true, many drugs are too poorly absorbed in the distal parts of the GI tract to be suitable for ER delivery (6). Poor colonic drug absorption rules out the likelihood of successful ER development, and if ignored, will result in costly development efforts that are carried out in vain. In standard bioavailability studies on MR formulations it is not possible to distinguish between poor formulation performance and insufficient active drug absorption. Therefore regional drug absorption should be assessed prior to embarking on an ER formulation development. The highest quality data is obtained by regional absorption studies in man, which will be more described in detail below. However, for NCEs it is desirable to evaluate regional absorption properties already in preclinical screening as part of the trigger for a decision to start product development. An example of a preclinical risk assessment scheme is given in Table 1. Some principles and test methods will be discussed in further detail below, including considerations of permeability, solubility, luminal degradation and gut wall metabolism as well as human study techniques.

Preclinical Regional Drug Absorption Assessment

The *permeability* classification of a drug according to BCS should, on the basis of theoretical considerations, be a very useful as a criterion for selecting a drug as an ER formulation. Classification of a drug as a low-permeability compound means that the drug is not completely absorbed after oral administration of a solution. For such compounds a certain amount of drug is clearly delivered to the colon after standard oral administration and the permeability in the colon must then be so poor that a significant part of the dose passes through the entire colon without being absorbed. This implies that the permeability in the colon is very low for such compounds, preventing significant drug absorption at that distal site. It has also been shown *in vitro* that the permeability of classes III and IV drugs is even lower in the colon than in the small intestine, whereas classes I and II drugs can sometimes show a slightly higher permeability in the colon when passive diffusion is the dominating mechanism (7). This permeability

TABLE 1 Preclinical Risk Assessment of Colonic Drug Absorption Prior To Embarking on Product Development

Level	Risk factor	Criteria	Implications
Green No critical factor identified	Good absorption over entire GI tract	BCS class I (high passive permeability/high solubility) <i>and</i> stable in colon fluid (e.g., <10% degraded in 1 hr)	Candidate for ER development
Amber Acceptable risk	Risk for poor colon absorption	High permeability after saturation/inhibition of efflux <i>or</i> intermediate solubility <i>or</i> degradation in colon fluid (e.g., >10% degraded in 1 hr)	Possible candidate for ER development but further evaluation recommended
Red Significant risk for development failure	Poor absorption from colon expected	Low permeability according to BCS <i>or</i> volume needed to dissolve max dose at pH 5.5–7.5 >20 L <i>or</i> rapid degradation in colon fluid (e.g., half-life <15 min)	ER development not recommended

Abbreviations: GI, gastrointestinal; ER, extended-release; BCS, biopharmaceutical classification system.

pattern has also been shown to be relevant for small and large intestinal specimens from humans when the Ussing chamber model is applied (8). Consequently, for low-permeability drugs it will not be possible to control the rate of absorption by an ER formulation—the inherently low rate of drug absorption would be the rate-limiting step. In addition, a large part of the dose will not be absorbed, leading to a low and variable bioavailability. This is further supported by a compilation of human regional absorption studies using remote control release devices and intestinal intubations. More specifically, it was found in studies of 11 low-permeability compounds that the relative bioavailability after colonic administration compared with oral or small intestinal delivery was generally between 0% and 50% (9), that is, the fraction of the administered dose absorbed in the colon would at best be about 25% assuming an oral fraction absorbed of about 50%. In contrast, high-permeability compounds according to BCS that were stable in colonic luminal contents had a relative bioavailability of at least 70% after colonic administration. Thus, permeability classification according to BCS, which can be estimated by computational modeling, in vitro or in vivo preclinical models, is a strong indicator for colonic absorption and feasibility of oral ER development when passive transcellular diffusion is the main mechanism for membrane transport. Compounds with low molecular weight (<200 d) that are highly hydrophilic, often have high small intestinal paracellular permeability and are not likely to be well absorbed from the colon because of the relatively small pore size of the tight junctions between the epithelial cells in this region (10). Similar behavior is expected for drugs having high small intestinal permeability because of uptake by active transporters, for example, peptide and amino acid transporters, which are not available in the colon (11). Finally, although the influence of efflux transporters on regional permeability and absorption remains to be further elucidated, in vitro data indicates that efflux activity decreases in the colon compared with the upper

small intestine (12,13). Thus, active efflux would not be expected provide a limitation for colonic drug absorption and ER delivery.

Solubility can be expected to limit drug absorption in the colon similar to the situation in proximal intestine. The situation in colon would be even more challenging since no solubilization due to bile acids would be expected in the colon: they are largely reabsorbed in the ileum. Lack of solubilization in human colonic fluid has been verified by drug solubility studies of felodipine. This aprotic drug has a water solubility of about 1 $\mu\text{g/mL}$, but it is extensively solubilized in FaSSIF, as indicated by an increase of the solubility to 40 $\mu\text{g/mL}$ in this media. However, the solubility in colonic fluid, 2 $\mu\text{g/mL}$, is very similar to the aqueous solubility (14).

Another factor which further limits drug solubility in the colon is the very small aqueous volume available for dissolution (see above). Dissolution limited drug absorption in the colon is strongly indicated by the many examples of incomplete drug absorption of BCS class II drugs as solid IR formulations. Despite the limitations arising from slow/incomplete drug dissolution there is still a potential for development of well functioning ER formulations for low-solubility compounds. For example, both felodipine and nifedipine, which have water solubilities of 1 $\mu\text{g/mL}$ and 10 $\mu\text{g/mL}$, respectively, are marketed as ER formulations (at doses of 10 and 120 mg, respectively) providing consistent drug absorption from both the small intestine and the colon (15,16). Formulation technology is most probably critical for successful development of such low-solubility compounds, that is, by combining ER drug delivery with solubility enhancing formulation principles. Thus, ER development of low-solubility compounds is clearly challenging, and will require more knowledge/better models to quantitatively assess dissolution limitations to colonic drug absorption.

Another limitation to colonic drug absorption is *degradation* by the large numbers of bacteria present through the entire colon. This is a specific feature of the colon since the presence of bacteria in the more proximal part of the intestine is negligible and most probably of no relevance for drug degradation. It has been suggested that the GI microflora has a metabolic potential equal to or even greater than that of the liver (17). There are, however, important differences between hepatic and bacterial metabolism. The liver is primarily responsible for drug metabolism via oxidation and conjugation producing polar metabolites, while the GI microflora is involved in reductive and hydrolytic reactions often generating nonpolar low-molecular weight degradation products.

The interest in colonic drug metabolism has been surprisingly low in the pharmaceutical area although the phenomenon has been well known for a long time. The significance of this factor is clearly shown by the fact that at least thirty marketed drugs are known to be substrates for these bacterial enzymes, including not only 5-ASA prodrugs for inflammatory bowel diseases designed to be activated by the bacteria in the colon but also others like digoxin, metronidazole, omeprazole and ranitidine (18). Drug degradation has been studied in a simple in vitro test at AstraZeneca during the recent years prior to candidate drug selection. Degradation mediated by bacteria has been obtained for about 40% of the tested drugs (51 drugs), implying that this phenomenon, if anything, is even more relevant for newer molecules than for established drugs.

It is of great relevance to assess this property prior to embarking on development of an ER product since a large fraction of the drug will be released in the colon for such a product and bioavailability of the active drug could

thereby be significantly reduced. There are examples in the past at AstraZeneca where this has proven to be important enough to stop development of a once-daily oral product. This is not surprising considering that the degradation rate, as determined by *in vitro* tests, can be very rapid, with some drugs exhibiting half-lives of less than 15 minutes. Drug degradation in the colon could also have implications for safety if the degradation product is more toxic than parent drug. These degradation products might also be further metabolized in the gut wall or liver, creating additional molecular species.

Colonic drug degradation can be studied by *in vitro* tests. The challenge is to obtain test systems reflecting *in vivo* metabolic activity, which is primarily determined by bacterial flora and amount of bacterial species. Different approaches have been described in the literature (18). Fermenters based on inoculum of fecal or animal colonic contents are used, either as a static system or by use of dynamic systems in which the growth medium is continuously added and old culture is removed. These systems are operated under anaerobic conditions, which is required for growth of some intestinal bacteria. Very sparse information is available in the literature regarding *in vitro/in vivo* correlation of such test systems. Therefore output from these *in vitro* studies has to be regarded as more qualitative than quantitative. Another approach is to study this degradation indirectly through *in vivo* bioavailability studies in animal models (18). This could be done by comparing biliary and fecal metabolites, studies in bacteria free animals compared with subjects with normal flora or by studies with and without extensive antibiotic treatment.

The final aspect that could influence regional drug absorption is differences in **gut wall metabolism** in different parts of the GI tract. It seems that the metabolic capability decreases along the intestine (see chap. 4). In contrast to the other factors discussed above, which limit use of traditional ER drug delivery, this factor could provide an additional rationale or benefit of ER delivery since delivery to the colon could reduce first-pass metabolism. For example, administration of oxybutynin in an osmotic pump tablet increased bioavailability of active drug with 50% compared with an IR reference tablet with a corresponding decrease in the main metabolite (19). In another study, the bioavailability of budesonide (a CYP3A4 substrate) was assessed by application via intubation to the colon and small intestine. When applied to the small intestine together with the CYP3A4 inhibitor, ketoconazole, the bioavailability increased two-fold. When coadministered with the same inhibitor in the colon the bioavailability was unchanged, demonstrating the importance of regional differences in gut wall metabolism (20). In addition, this further highlights the possibility of reducing drug-drug interactions when an MR formulation delivers the main part of the drug to the colon.

Gut wall metabolism has so far been shown to be important only for cytochrome CYP3A4/CYP3A5 oxidative metabolism and phase II conjugation reactions and thus it is only in those cases that regional difference in first-pass metabolism need to be considered (21,22). Gut wall metabolism is addressed in much more detail in chapter 4.

In Vivo Human Study Techniques

It is desirable to obtain information about the bioavailability of a drug in different regions of the GI tract prior to formulation development. Different

experimental techniques are available. The most common are remote control capsules and oral intubation techniques or colonoscopy (6,23).

For the latter, the drug must be dissolved or suspended in a small volume, which is housed in a chamber or balloon in the remote control device. The location of the device in the GI tract is determined by fluoroscopy or gamma scintigraphy. When the target location has been reached, the drug release mechanism is triggered externally, for example, by radiowaves, and the drug is released in the intestinal fluid as a bolus dose.

When using intubation, the tip of the tube is moved to the desired location in the GI tract by the motility and the drug is administered as solution or suspension. The position of the tube is determined by fluoroscopy or scintigraphy before administering the drug, analogous to the remote control devices. The use of intubation allows multiple administrations as well as continuous drug infusions over a longer period, providing an input rate more similar to that of an ER formulation.

Both types of techniques have been shown to provide very valuable results, but certain pros and cons can be identified. For example, multiple doses are possible, and the rate of drug administration can be varied in the case of intubation, thus providing an input rate similar to an ER formulation. This is presently not possible for the remote control devices. Further, the potential risk of not obtaining appropriate drug release at the desired site is lower for intubations, owing to its simplicity, as compared with the more highly technological remote control devices. On the other hand, the tube or the perfusion may perturb the normal physiological flow conditions in the intestine.

The bioavailability after administration in more distal parts of the intestine, such as the terminal ileum and different parts of the colon, is compared with a reference administration, either as an oral solution or as a regional delivery to the upper small intestine. For example, regional administrations of metoprolol provided not only the same extent of absorption but the plasma concentration-time profiles were almost superimposable, indicating that the rate of absorption was also the same (23). By administering the drug both as solution and as solid particles in the GI regional administrations, it is further possible to distinguish dissolution limitations from other factors limiting absorption. Such information can have great implications for formulation design. A study design similar to the one used for metoprolol was applied to a low-solubility compound (30 µg/mL) aimed for colonic delivery. No significant difference in AUC was obtained in this case between a suspension and a solution of the drug administered to the colon. These data strongly suggested that there was no need for solubility enhancement to obtain a well functioning ER product.

IN VITRO SCREENING OF MODIFIED-RELEASE FORMULATIONS

MR formulations are often designed to provide a controlled rate of dissolution over many hours to optimize drug exposure at the site of pharmacological action and thereby improve clinical efficacy and tolerability. Dissolution testing reflecting the *in vivo* release and dissolution is therefore a key test to achieve and maintain desired clinical properties.

Historically, *in vitro* dissolution testing has been strongly associated with development of one method for quality control. However, to support ER

product development, a dissolution test strategy typically includes several tests. In vitro dissolution is used in product development to select and optimize in vivo properties of prototype formulations during initial phases of product development. The main aim is to predict in vivo behavior, often defined as a mean in vivo profile obtained under physiological conditions, for example, corresponding to fasting state in the “average” healthy volunteer. However, an additional aspect of in vivo predictive dissolution testing is to assess the risk for deviations in in vivo dissolution potentially leading to adverse events or poor efficacy due to changes in drug release from the ER formulation resulting from physiological variations in the GI tract. The aim of dissolution testing in the later stage of product development is to document or support equivalence regarding in vivo performance between different clinical trial formulations/commercial product obtained after manufacturing scale-up and optimization as well as aspects in designing a quality control test or a clinically relevant design space according to quality by design principles (see also chap. 19).

This chapter will describe considerations in designing in vivo predictive dissolution tests, both initially prior to availability of relevant in vivo data and later refined on the basis of bioavailability data. Factors to consider regarding in vivo robustness dissolution testing will be addressed separately. Other useful information regarding design and performance of in vivo relevant dissolution tests can be obtained in *Pharmaceutical Dissolution Testing* edited by Dressman and Krämer (24) and chapter 13 of this book.

In Vitro Dissolution Testing in Selecting and Optimizing Prototype Formulation

How can an in vivo relevant method be defined in an ER product development project prior to existence of any in vivo data? This is a question all product development teams are facing. This section provides a scientific background to addressing this task.

There are two principal ways to achieve in vitro tests that can predict in vivo results, either by complete mimicking of the GI tract conditions or total robustness of drug dissolution from formulation to the normal variations in physiological conditions along the GI tract (e.g., pH, fluid volume, motility). In the former case, there are attempts described in the literature and available for commercial use that aspire to capture the dynamics of the GI tract (25). Although such approaches represent a step forward in biorelevant dissolution test methods, they are still very far from ideal. For example, hydrodynamics force, fluid volumes and colonic conditions are factors that are not fully addressed in an in vivo relevant manner and this is an important area for more research and development in the future. The other option is to use an ER formulation principle that provides the same dissolution profile irrespective of conditions for dissolution. Here even simple standard dissolution testing would provide in vivo relevant results. For example, the drug dissolution from coated beads of metoprolol succinate was very insensitive to different pH, agitation, etc., and a 1:1 relation between in vitro and in vivo dissolution was obtained by use of a simple standard dissolution test (26). However, this is not the case for most ER products, because some molecules provide challenges, like poor solubility, which preclude existing ER technologies from providing

TABLE 2 Examples of Factors Influencing Dissolution In Vitro and In Vivo for Different Types of Release Mechanisms

Release mechanism	Common factors influencing drug dissolution in vitro and in vivo
Diffusion layer coatings	Drug solubility, buffer capacity, osmolarity
Diffusion matrices	
Osmotic pumps	Osmolarity, drug solubility
Swelling controlled hydrophilic matrix	Osmolarity, fluid components—matrix polymer interactions, pH solubility profile of matrix polymers
Erosion-controlled hydrophilic matrix	Hydrodynamics, osmolarity, fluid components—matrix polymer interactions, enzymatic degradation of matrix forming polymer
Erosion-controlled hydrophobic matrix	Hydrodynamics, solubilization of matrix forming agent
Slow dissolution from drug particles	Drug solubility, hydrodynamics
Enteric coating	Coating polymer solubility, buffer capacity, ionic strength

this kind of robustness. Therefore, a more pragmatic approach often needs to be taken in dissolution testing as a part of product development. The basis for designing an in vitro test that provides in vivo relevant results requires consideration of the following factors:

- Drug substance solubility properties
- Understanding of the release rate controlling factors of the ER formulation
- GI physiological conditions, for example, fluid composition/physical chemical characteristics, GI residence time and hydrodynamics conditions

The combined understanding of these factors allows identification of critical aspects of the design of the dissolution test with respect to in vivo predictability. The key GI physiological aspects for consideration are summarized in the preceding section. The most common release principles and example of dissolution influencing factors of special relevance to consider in design of a dissolution test are summarized in Table 2.

In reality, the release from an ER formulation can be controlled by multiple mechanisms or it could change depending on the test condition. For example, the switch of pH from acidic conditions to neutral could alter release of a basic compound from swelling to erosion controlled for a hydrophilic matrix tablet. Clearly, some in vitro dissolution experiments need to be designed to verify the release mechanisms that are hypothesized initially on the basis of mechanistic models and past experience. Complementary techniques to simple drug assay have been applied in in vitro dissolution testing during the recent years to support a mechanistic understanding of the drug release. These include the following:

- Dissolution of key excipients (27)
- Particle size analysis, for example, by Coulter counter (28)
- Formulation morphology investigations by NIR (29)
- Drug solid state analysis, for example, by Raman spectroscopy (30)
- Imaging by MRI following size changes and water concentration profiles (31)
- Mechanistic based modeling of in vitro dissolution data

In the early phase of product development, one or more dissolution tests should be designed to take into account the expected relevant factors. Different aspects of these considerations will be exemplified below.

Drug solubility is a key aspect for formulations based on diffusion through coatings or porous matrices, since the driving force for release will be created by the concentration gradient between the inside and outside of the formulation. This will clearly be dependent on the drug solubility, further modulated by composition of coating or matrix layers. For high-dose formulations where the active drug constitutes the main component, drug solubility could also affect the erosion of matrices and thereby the drug release. Finally, for more poorly soluble drug substances, the actual drug particle dissolution could provide an ER profile without additional ER principles. However, this is a rarely used and is not a recommended approach since it provides very poor control of *in vivo* dissolution. Thus, a prerequisite to obtain *in vivo* relevant dissolution profiles for such cases would be to design *in vitro* tests that reflect the drug solubility in the relevant parts of the GI tract.

A key determinant of drug solubility for ionic drugs is the pH. Since the pH varies along the GI tract, a pH shift method may be needed in which the pH can be changed to correspond to the situation in the GI tract. If a simple two-step method is deemed to give sufficient *in vivo* prediction (stomach acid pH + close to neutral intestinal pH) this can be obtained by adding a strong buffer to the originally acidic solution. Final ionic strength/osmolality should be considered in this two-step approach if the formulation performance is sensitive to these latter factors. When multiple media are needed, it could be useful to use alternative dissolution methods, like USP apparatus 3 or 4, which either automatically move the formulation from one medium to another (USP 3) or allow simple switch of medium (USP 4). With both methods, media can be tailored to the expected GI transit times. More elaborated pH shift methods may however be needed and one example has been described in the literature that allows multiple change of pH to more closely mimic the *in vivo* situation (32).

In vitro test systems rarely use the same buffer components as available in the GI tract since the carbonate buffer available in the intestine forms carbon dioxide in the open air and thus does not provide a stable pH (33). This could be of importance since different buffers could alter drug solubility at the same pH, for example, because of formation of low-solubility salts with buffer species, salting in/out phenomena or variation in buffer capacity. Therefore, some validation is recommended regarding the selected buffer, at least by confirming similar results for different buffers to detect anomalies. The buffer capacity may merit further attention since this factor could affect the microclimate within the formulation. Very high concentrations of dissolved drug and excipients can be obtained locally within the formulation and the buffer capacity will be a critical determinant of the internal pH and thereby drug solubility and release. In contrast, the buffer capacity in the bulk of the test media will be less of a concern since much lower concentrations of drug and formulation components will be available with negligible influence of pH—as long as plain water is avoided. The buffer capacity of the often-used 0.1 M phosphate buffer pH 6.8 is 20 mmol/L/ Δ pH, four times higher than the buffer capacity in human intestinal fluid in the fasted state (34). The buffer capacity of simulated fasted intestinal fluid according to Dressman (35) is also higher than human intestinal fluid, though to a much lesser degree than the standard phosphate buffers. The

TABLE 3 Buffer Capacity of Different Dissolution Media Together With In Vitro Dissolution Rate for a Diffusion and an Erosion-Controlled Matrix Extended-Release Tablet of a Basic Drug and In Vivo Dissolution Rate^a in Human Determined from Plasma Drug Concentrations

Medium	Buffer capacity (mmol/1/ Δ pH)	Drug dissolution rate (%/hr)	
		Durules	HPMC
0.1 M phosphate buffer pH 6.8	20	8	7
FaSSiF	10	7	7
<i>Human intestinal fluid</i>	5	3 ^a	7 ^a
Modified FaSSiF	4	2	6

Modified FaSSiF, FaSSiF with reduced phosphate buffer concentration.

importance of this factor is illustrated in Table 3. The dissolution of a basic drug with pK_a below intestinal pH was studied from one diffusion and one erosion-controlled matrix ER formulation in different media and compared with the dissolution in vivo calculated by deconvolution from plasma concentration-time data. The buffer capacity did not influence the drug dissolution from the erosion-controlled system and there was a good correspondence to in vivo data, whereas for the diffusion-controlled matrix, standard buffer or FaSSiF provided too rapid dissolution compared with the in vivo situation. A much better correspondence between in vitro and in vivo was obtained after lowering the buffer capacity of the phosphate buffer to levels more closely resembling the in vivo situation. A more sophisticated approach to obtain in vivo relevant buffer capacities has recently been proposed by Sheng et al., allowing theoretically based calculations of suitable phosphate buffer concentrations using drug solubility and pK_a (36).

Most of the considerations regarding drug solubility would also be applied to dissolution of critical excipients with pH-dependent solubility. This would primarily be coating or matrix forming excipients. Examples of such pH-dependent components are cellulose derivatives and methacrylates used for enteric coating.

For low-solubility drug compounds, the influence of solubilization through bile acid micelles need to be considered similar to the situation for IR formulations. An additional aspect for ER products in this respect would be drug dissolution in the colon. The bile acids are reabsorbed at the end of the small intestine and the amount leaking into the colon is too low to effect solubilization. In addition, the volume of fluid in this area is restricted to pockets of small volumes. Thus drug particle dissolution could be expected to be a limiting factor for drug dissolution from an ER formulation in the colon for low-solubility compounds. This has been strongly indicated for a low-solubility drug (felodipine 1 μ g/mL) in an ER hydrophilic matrix tablets, from which the drug release in standard dissolution testing under sink conditions as well as in the stomach and small intestine is controlled by tablet erosion (37). A small study was conducted with three different matrix tablets with same quantitative composition but including the drug in three different forms, amorphous, crystalline micronized and crystalline coarse grade particle size (data on file, AstraZeneca R&D). All tablets had the same in vitro dissolution-time profile

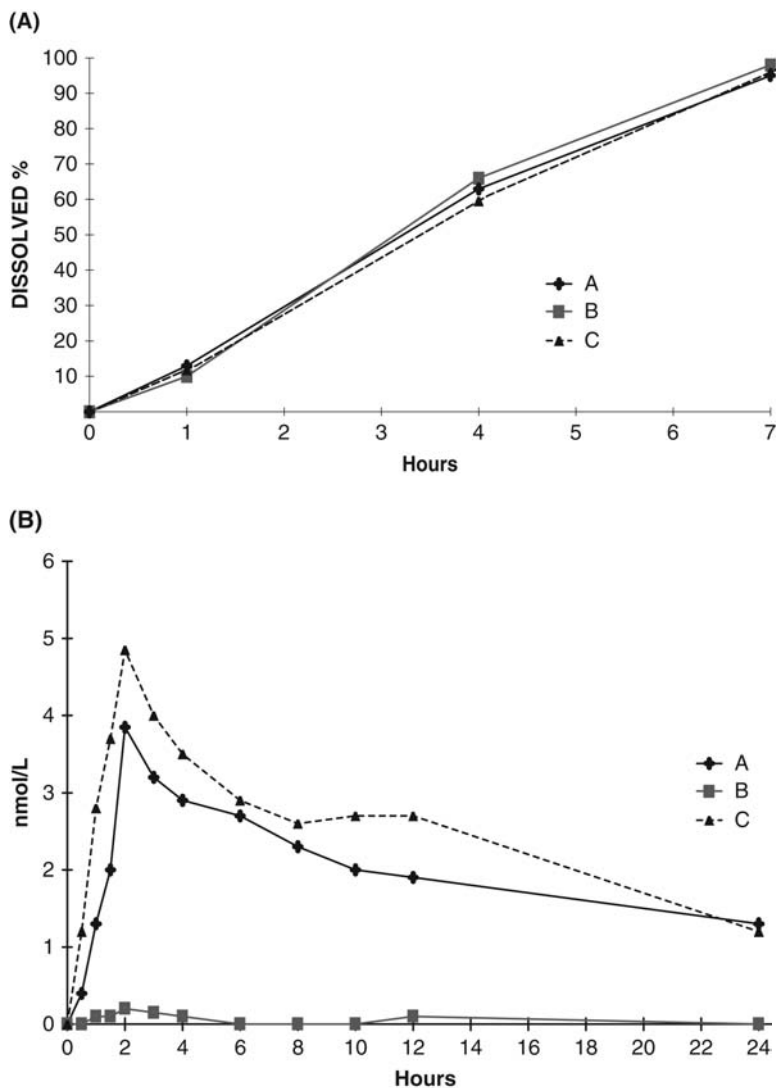


FIGURE 5 (A) Mean in vitro dissolution ($n = 6$) and (B) mean plasma concentrations ($n = 3$) of felodipine extended-release hydrophilic matrix tablets with same quantitative composition but different active drug particle forms: crystalline micronized (A); crystalline coarse grade (B); and amorphous (C) (AstraZeneca R&D).

using the USP product monograph method for felodipine ER tablets (Fig. 5A), not unexpectedly, since drug dissolution was controlled by tablet erosion for all three tablets. But the bioavailability differed profoundly (Fig. 5B). The very low bioavailability for the coarse drug formulation could certainly be explained by the slow dissolution rate of the felodipine particles, that is, the tablet erosion was no longer the rate controlling mechanism for drug absorption. This change was

not at all reflected by the dissolution test performed at sink conditions. Unfortunately, there are not yet any test conditions that represent the colonic conditions in a realistic manner.

Other important factors are the osmolarity and ionic strength of the test medium. They are interrelated since an increase of the ionic strength through buffer components also will increase the osmolarity. These factors could influence functionality of most polymers used for ER formulations with potential consequences for the drug dissolution. For example, the gel strength of nonionic cellulose based hydrophilic matrices would initially be increased, leading to slower matrix erosion, when the osmolarity/ionic strength is hypertonic (38). Eventually the gel will collapse through complete dehydration and the system will then rapidly disintegrate, leading to dose dumping. This effect varies with the presence of salts according to the Hofmeisters lyotropic series according to which phosphates, which are often used *in vitro*, have much stronger effects than chloride (39). The importance of this factor for *in vivo* predictions of eroding hydrophilic matrices was shown in an IVIVC study in which tablet erosion was measured *in vitro* and *in vivo* for two different formulations (40). The selection of phosphate buffer ionic strength proved to be critical to obtain *in vivo* predictive results. Surfactants, often included in dissolution media to increase solubility of low-solubility compounds, influence gel strength in a similar manner (41). It was found in an IVIVC study that nonionic or cationic surfactants are more favorable in this respect than sodium dodecyl sulfate (SDS), which is considered to be the surfactant of choice in dissolution testing (42). The use of SDS led to poor discrimination between matrices containing different qualities of hydroxypropylmethyl cellulose, even though these clearly provided different *in vivo* performance. The issue was resolved by changing to a cationic or nonionic surfactant.

Systems that release by osmotic pumping would also be counteracted by a high osmolarity in the dissolution medium (43). The GI fluids in the fasting stomach and small intestine are hypoosmotic (34) whereas food intake can alter the osmolarity in the stomach. There are some indications that hyperosmolarity can be achieved in the initial part of colon, probably because of high concentrations of organic acids liberated from fibers digested by the colonic bacteria.

Perhaps the most challenging aspect of designing *in vivo* relevant *in vitro* dissolution test methods is the hydrodynamics. The GI hydrodynamics are of a complex and heterogeneous nature. Clearly, there is not yet any dissolution apparatus that captures the hydrodynamics in a meaningful way. There are two aspects that are important to ER formulation performance: fluid motions that generate forces on tablets tangent ("shear stresses") and perpendicular forces ("crushing force"). The former varies substantially with position of the tablet in relation to motility waves and could affect erosion rates (44). Crushing forces occur especially at the sphincters, that is, pylorus and the ileocecal junction as well as during maximum amplitude of a motility wave, for example, in the antral part of the stomach.

Some attempts have been made to model GI shear forces indirectly by correlating *in vitro* dissolution and *in vivo* bioavailability data, for example, by varying stirring velocity in a USP apparatus 1 or 2 to mimic release profiles measured *in vivo* (40,45). However, these results are very difficult to generalize because of formulation-specific hydrodynamic effects *in vitro* as well *in vivo*, for example, due to density and shape. A more rational approach to modeling shear

forces in the fed stomach has been developed (44) using a combination of computer simulation and *in vitro* experiments. From two separate computer models of tablets in the fed stomach and of tablets *in vitro*, the intragastric range of surface stress and Reynolds number was estimated, and then a dissolution apparatus (rotating beaker with tablet in a fixed position) and parameter space was designed to replicate the *in vivo* conditions. These simulation studies identified a range of shear forces dependent on tablet location from the “no-shear” situation in the fundus to very high transient levels occurring when the tablet was forced in the reverse direction through advancing antral contraction waves (46).

Crushing forces in the upper GI tract have been studied *in vivo* by Kamba et al. by developing tablets with different pressure-sensitive disintegration and drug release (47). They later measured the corresponding destructive force at different places and agitation intensities in the USP paddle apparatus as well as the disintegration apparatus, providing some suggestions about how to do *in vitro* studies under conditions more relevant to *in vivo* (48). It has also been proposed to mimic these forces by using a texture analyzer in combination with dissolution testing which allows applying a certain force on the tablet surface (49). However, this is a cumbersome method requiring further evaluation and development to become practically useful. Clearly, there is still a need for increased understanding and design of new *in vitro* methods better capturing the varying hydrodynamic effects of the GI tract.

Besides the desire to create *in vivo* relevant hydrodynamics, *in vitro* artefacts must be avoided. For example, the flow rate is substantially different in the USP 2 (paddle) method depending on the position of the formulations (50). Thus, for formulations sensitive to this factor, greatly varying results are obtained merely by changing the formulation position in the vessel. Visual inspections of dissolution experiments could be very helpful to detect such factors, which could be documented by video recordings. High-density formulations will stay at the bottom of the vessel below the paddle where agitation is very poor, and the reverse situation can occur for low-density formulations, which will float on the surface of the test medium. Another issue is hydrophilic gel matrix tablets, which could randomly stick to the vessel wall because of their adhesiveness, leading to very variable dissolution results. These kind of hydrodynamic artefacts should be avoided, for example, by use of modified paddle methods (15), sinkers or alternative apparatuses, primarily the USP 1 (basket) method. It should be noted that with the use of such devices dissolution still could be affected by orientation of the dosage form within the basket or sinker, leading to high variability or artefactual differences between formulations.

Regulatory requirements on dissolution testing state that testing should be performed at mild agitation for IR formulation, that is, 100 rpm in USP 1 and 50 rpm in USP 2 (51). This is also required in practice by regulatory authorities for MR formulations unless variability or IVIVC data can be used to justify more intense agitation. This is unfortunate since there is no clear scientific rationale or supporting data regarding that lower agitation should be more discriminative for MR formulations. On the contrary, too low agitation mainly increases the risk for *in vitro* artefacts leading to misinterpretations of data.

It is clear from the considerations presented above that an *in vitro* test strategy and design of methods have to be considered for each drug development project. One consequence is that the generic MR product developer has

hardly any benefit from using originator company QC methods published through USP and FDA websites. Instead, they need to establish their own methods. Another consequence is that a rational approach to dissolution testing aiming for in vivo predictions benefits from applying the best of our scientific understanding. The relatively slow progress in the area and many examples of poor IVIVC can be attributed to too simplistic trial and error approaches where underlying causes for both in vitro and in vivo data have been neglected. Finally, the science involved is multidisciplinary, meaning that developing dissolution methods which are predictive of in vivo behavior not only needs involvement from analytical chemists for robust drug assays but also from formulation scientists for understanding of formulation mechanisms, from biopharmacists for understanding of the in vivo drug absorption limitations and formulation versus GI physiology interactions as well as from clinical pharmacokineticists for interpretation of in vivo data.

Refining Biorelevant Dissolution Methods Based on In Vivo Data

The initially defined dissolution tests should be validated and refined when in vivo data for prototype formulations become available. Preliminary IVIVC could in theory be established by use of animal PK data, but that would require careful considerations of the relevance of the animal model for man. More definitive IVIVC based on refinement of an in vitro predictive test requires human BA data. One important consideration in refining an initial in vitro method to improve IVIVC is to avoid adjustment of the in vitro conditions to PK/absorption effects other than drug release/dissolution. When preceding regional absorption studies have indicated local differences in absorption, a simple 1:1 correlation could not be expected. Instead, additional mathematical modeling linking in vitro data to plasma concentrations would be required as exemplified by work from Balan et al. (52). A similar situation would occur for drugs having nonlinear pharmacokinetics (note that local concentrations at enzymes would be much lower after MR administration compared with corresponding doses given as IR formulations). This could either be performed on a purely empirical basis using linear or polynomial functions, or more mechanistically based by use of more advanced absorption simulations software (53). Another important aspect in refining dissolution methods on the basis of in vivo data is to keep settings within boundaries that at least potentially could be in vivo relevant. The primary variables to adjust would be those that are less well known in vivo, like agitation intensity, with perhaps additional fine-tuning of media composition and volume within physiologically relevant limits. Less in vivo relevant approaches, for example, use of cosolvents and nonphysiological pH, to establish IVIVC brings a much higher risk of poor prediction when applied outside the exact formulations that have been studied to establish the IVIVC. Finally, it is rarely useful to include formulations utilizing different release mechanisms in the same IVIVC because the influence of different factors would often differ between mechanisms (42,54–56). Thus IVIVC should generally be regarded as specific for a certain combination of drug and formulation principle. For formulation principles where drug properties (e.g., solubility) do not affect the dissolution profile, like eroding matrix formulations, more generic IVIVCs might be possible.

Additional refinements of IVIVC methods might occur in an iterative manner when more data and understanding are generated during the development process.

In Vitro Testing of Dissolution Robustness Vs. Physiological Factors

Most dissolution testing aims to predict an “average” in vivo profile in a typical patient, often modeled in bioavailability studies by healthy young subjects. However, there is a variation in the physical and physicochemical conditions in the GI tract both within a normal population and due to disease or age factors (see chaps. 6 and 7). It is desirable in selection of an MR prototype for further development to attain a certain level of robustness of drug release properties to achieve consistent clinical product properties. The greatest concern is the risk of dose dumping, that is, the ER release control is destroyed at some point and all drug is rapidly released similar to an IR formulation. No or very poor release, leading to subtherapeutic effects, is also theoretically a concern but there are fewer reports and concern regarding such events.

Factors to be considered for inclusion in physiological robustness testing include the following:

- Physicochemical/physical factors covering the entire possible range in the GI tract.
 - *pH* 1 to 8.
 - *Agitation* tested, for example, by changing beaker or paddle rotation (USP I and II), dip rate (USP III), flow rate (USP IV) or addition of beads to the USP II method (57).
 - *Osmolarity/ionic strength*: Osmolarities between 100 and 800 mOsm/L may transiently be obtained in the gut.

The intake of food and fluids could introduce additional effects beyond pH, agitation and osmolarity effects discussed above. For example, it has been shown that food components may prevent dissolution from solid formulation because of adherence of lipids or formation of films consisting of precipitated proteins (58). This effect is most probably not a major concern for ER formulations since such effects are most often marginal compared with the built-in slow release properties of the formulation and would only influence initial dissolution rate, but other interactions might occur which could merit testing with food components. Intake of ethanol is another factor that has recently attracted great attention because of reports of dose dumping which in turn led to serious clinical events (59). Ethanol could strongly alter functionality of some polymers used in MR formulation by increasing or reducing solubility of the polymer. For example, the individual peak plasma levels of morphine were increased up to 16 times after intake of an ER tablet together with alcohol, because of dose dumping (59). In addition, ethanol could also increase solubility of low-solubility drugs potentially increasing rate of dissolution. A preliminary in vitro test strategy has been proposed including testing at 4%, 20%, and 40% ethanol (60).

It should be noted with respect to food effects that there is a great potential for other effects by food, beyond changes in drug release/dissolution leading to a change in plasma concentration, and that these would consequently not be

possible to predict by dissolution testing. Food effects on first-pass metabolism or clearance are well known, but other effects specific for MR formulations are also possible. For example, if there are regional variations in drug absorption, for example, reduced bioavailability in the colon, the longer residence time in the stomach induced by food will increase the overall extent of absorption. Another factor could be poor mixing in the fundus of the fed stomach, which can lead to accumulation of the released drug in the stomach. When later emptied into the small intestine, this generates a plasma peak, a kind of “physiological dose dumping” phenomenon not directly related to effect on drug dissolution (37).

Preclinical Models

In vivo studies using preclinical models would rarely replace studies in human but could provide useful information to

- establish an early IVIVC for in vitro dissolution tests,
- support selection of prototype formulation for further development especially when there is great uncertainty around in vivo predictive capability of in vitro methods,
- reduce risk of BE failures between clinical trial formulations and/or commercial formulations in late development, and
- investigate mechanism of release, for example, using intestinal sampling/imaging.

The advantage of in vivo models compared with in vitro testing is that they capture the complexity and dynamics of critical factors in the GI tract influencing drug release and absorption. The main limitation to the usage of animal models is that no single species resembles all physiological properties of man. This introduces a risk that the results obtained in the animal model are not fully predictive for the situation in man. In the evaluation of oral dosage forms, the main aspects to consider are physiological features of the GI tract, such as dimensions, residence times in different segments, motility patterns, secretions, physical and physicochemical characteristics of GI fluids, the presence of enzymes that could metabolize drugs, and critical excipients, since these factors could directly affect the formulation performance. These factors can be handled by choosing the best possible animal model and an appropriate study design and by integrating knowledge of differences between animal and human in the interpretation of obtained results. Considerations in selecting and interpreting data will be further exemplified below. Differences between animal and human of other pharmacokinetic factors such as first-pass metabolism, clearance and volume of distribution are less of a concern since the in vivo studies should be designed as relative bioavailability studies—comparing different MR products, using a reference formulation to enable estimations of in vivo dissolution profiles, or as a robustness test versus food effects.

This chapter will focus on the use of the dog model since this is by far the most commonly used species. In many cases, it is an acceptable model because of its similarity to human regarding anatomy, motility pattern, residence times and many secretory components (61). It should, though, be cautioned that different breeds of dogs are used in such studies, which may impact results. For example, intestinal volumes and dimensions will differ significantly between a

fairly small beagle dog and a bigger Labrador. Some comments will also be included for the minipig and pig, which, although being less well characterized than the dog, have been proposed as a relevant model for such studies. The present discussion is not aiming at a comprehensive comparison of physiological aspects of relevance for MR delivery but more at providing a few considerations of specific relevance for testing of MR formulations. More general comparative reviews of GI physiology for animal models and human can be found elsewhere (61–63).

A prerequisite for ER studies in preclinical models is that the regional GI absorption of the active drug is not different to human in a way that would confound interpretation of results. The dog model seems to be a very good model from this perspective since there is a very strong correlation to human in terms of relative bioavailability after colonic drug administrations (64).

A concern often raised for the dog model is the shorter colon compared with human, leaving less time for absorption from a long-acting ER formulation. However, in our experience with larger dogs (20–35 kg) using X-ray imaging of labeled formulations, single-unit tablets usually remain in the gut for at least 24 hours. For comparison, it should be noted that the residence in the GI tract in human of healthy subjects can, in some cases, be substantially less than 24 hours (65).

Another aspect relating to GI transit is the risk of prolonged gastric retention of larger single-unit formulations in dogs. This has resulted in overly positive interpretation of dog data obtained for gastro-retentive formulations, that is, the gastro-retentive properties were not confirmed in humans (66). This factor might also be dependent on the size of the dog model.

Gastric emptying of single units as well as liquids has been shown to correspond well to human in domestic pigs, so this would theoretically be a better model in this respect (67). From a practical point of view however, minipigs are a more feasible model than domestic swine because of their smaller size and GI dimensions, both being closer to man. However, despite this similarity it has been shown that fairly small sized nondisintegrating tablets (6 mm) do not empty out of the stomach of minipigs that have been fasting for 20 hours (68). The reason for the gastric retention in smaller pigs could be the *torus pyloricus*, a unique constriction to the swine pylorus, which may affect the gastric emptying time of solid formulations. The minipig would thus not be considered a relevant model for studies of single-unit ER formulations unless focus is on performance in the stomach.

One of the most well-known differences between dog and human is the poor basal gastric secretion in dogs, leading to a variable gastric pH between 1 and 6. This would of course be of relevance for pH-dependent drugs and polymers. One strategy to circumvent this variability is administration of pentagastrin or hydrochloric acid prior to drug administration to better simulate the human fasting state (69,70).

There is a continuous effort within the industry to reduce, replace and refine animal studies. Thus, in the future, less animal studies will be required and more of the screening for MR products would be conducted on the basis of in vitro dissolution, computer simulation and prior knowledge. The remaining animal studies would also be designed and performed at an even higher knowledge level, providing maximal information from such in vivo studies.

IN VIVO STUDY METHODS

Design and Evaluation Aspects of Bioavailability Studies

The purpose of conducting human bioavailability studies with MR formulations varies through the development process. At an early stage of the product development, the main purpose may be to screen different MR principles to assess the feasibility of the MR concept and to decide which MR principle to develop further. Following this initial stage, there may be a need to optimize the MR formulation with respect to drug release profile, bioavailability, interactions with food, drug load, etc. At this stage a bioavailability study is required for an *in vivo* evaluation of the optimized formulation(s). These kinds of bioavailability studies clearly have a screening purpose and several different formulations are likely to be tested in each study. As a result of the screening of MR principles and drug release characteristics, a formulation ready for scale-up is obtained. Following the scale-up activities a bioavailability study may be required with the purpose of confirming that the *in vivo* performance of the MR formulation is maintained. The preferred timing of this bioavailability study is after scale-up to commercial scale but prior to the first pivotal study. In particular, if the MR formulation is changed in any respect between phase IIB and phase III, a confirming or bridging study is recommended. Finally, another type of bioavailability study may be done during late development, for instance parallel with the clinical phase III program, to develop an IVIVC for regulatory purposes. This is done to facilitate specification setting, justify *in vitro* dissolution methodology, support biowaivers for late stage changes of the formulation composition or manufacturing process and/or to define clinically relevant design spaces for manufacturing. In addition to these common bioavailability studies, more specialized human studies may be conducted. Examples are studies employing fractionated dosing of solutions or suspensions to simulate drug delivery from a MR dosage form, regional absorption studies by use of special devices and mechanistic imaging studies of formulation GI transit and *in vivo* drug release. Bioavailability studies supporting MR product development might also include measurement of pharmacodynamic markers to validate PK/PD models and confirm suitability for that kind of delivery profile (1).

Design

The study design depends on whether the bioavailability study is conducted for screening, confirming or IVIVC reasons. Furthermore, critical product properties influencing the study design may have been already identified at an early stage. For instance, if absence of food interactions has been identified as a crucial property of the MR product, the design of the early screening studies should comprise both fed and fasting study arms.

Common to all bioavailability studies is that they are most informative and conclusive if administration of a reference is included in the study design. Such a study design facilitates the comparison of different formulations both within and between studies and allows determination of relative bioavailability in each study subject as well as estimation of the drug absorption profile.

The reference could be widely different depending on what kind of information is required from the study. In the early screening studies, it is important to assess the bioavailability and to estimate the drug absorption time profile. An appropriate reference would in this case be an oral solution or an IR

tablet of the drug, or possibly an IV formulation. For such a reference a crossover study design is recommended, with one group receiving the MR formulation and the other receiving the reference. An alternative approach is to use isotope-labeled drug in the reference formulation, which allows simultaneous administration of the MR formulation and reference, thus avoiding a crossover study design. The labeling could either be a stable isotope such as ^2H or ^{13}C , or a radioactive isotope such as ^{14}C (71). The beauty of the isotope label approach is that the variability is reduced to a minimum since all intraindividual variability is eliminated. In addition a more simple study design can be used. Possible drawbacks are the more complicated manufacturing processes, both of the labeled drug substance and the reference formulation, and that for studies with stable isotope-labeled drug, bioanalytical methods separating labeled and non-labeled drug substance need to be developed. The methods for detection of stable isotope-labeled drug generally have similar quantification limits as the methods for nonlabeled drug. Thus, to reach concentrations in plasma or blood that are sufficiently high to allow pharmacokinetic evaluation, the dose of the stable isotope-labeled drug needs to be in the same range as the dose of the nonlabeled drug. Consequently, if there are any safety concerns, or if the pharmacokinetics are nonlinear, the stable isotope approach may not be possible. The analytical methods for radiolabeled drugs are much more sensitive allowing significantly lower doses of the reference, sometimes referred to as micro or tracer doses. Recently, a new approach has been proposed using advanced analytical techniques such as accelerator mass spectrometry (AMS) or positron emission tomography (PET) allowing for microdosing of labeled drug substance (72) which could be very useful for reference administrations in MR bioavailability studies.

The purpose of a confirming or bridging bioavailability study is to verify that a change of the composition or manufacturing process, for instance a scale-up, has not significantly altered the *in vivo* performance of the formulation. For these studies also the reference could be chosen as described above, but it is probably more appropriate to use the unaltered MR formulation as reference.

The purpose of an IVIVC study is to develop a correlation between the *in vivo* performance and the *in vitro* drug release of the MR formulation. The most informative and useful correlation, known as a level A correlation, is a point-to-point relationship between the *in vitro* drug release profile and the absorption profile. However, to establish clinically meaningful specification limits and design spaces for manufacturing, level C correlations (average bioavailability variables vs. *in vitro* dissolution rate) should be sufficient. Such correlations then need to be established for all variables reflecting clinical effects, like peak and trough plasma concentration levels and AUC. Definition of dissolution rate intervals in which the AUC maintains unaffected could also be useful in defining dissolution specification limits. To develop such correlation, the IVIVC study should comprise a reference similar to that in the screening bioavailability studies.

There are numerous examples of MR formulations for which concomitant intake of food affects the clinical performance. To reveal if there are any interactions with food and to assess the degree of impact of food on the *in vivo* performance of the formulation, dosing under fed conditions should be included in the study design. The standard and most simple design is a two-way, crossover design comparing administration under fasting and fed conditions. The standardised FDA breakfast is usually given in the fed arm, but

alternative compositions of the meal can also be considered. The food is normally given 30 minutes prior to administration of the study formulation. It could also be very informative to time the food intake differently, for instance administer the study formulation immediately before start of food intake to secure the placement of food above the study formulation in the stomach.

Depending on the degree of food interactions and the need for controlling them, fed state administrations could be included in the screening and confirming bioavailability studies.

Evaluation

Crossover study designs are preferred since these enable intraindividual comparisons, for example, between the study formulation and reference or between fed and fasted administrations.

Primary variables to evaluate are usually maximum drug plasma concentration, $C_{\max}$, area under the drug plasma concentration-time curve, AUC, and time to reach $C_{\max}$, $T_{\max}$. From these primary variables other parameters can be calculated, for example, relative bioavailability or relative effects of food. In addition, the drug plasma concentration at a time-point corresponding to the intended dosing interval (τ), typically 12 or 24 hours, could be included in the primary variables. For instance, the drug plasma concentration at 24 hours would correspond to the trough concentration for a 24-hour dosing interval. The $C_{\max}/C_{t=\tau}$ ratio is indicative of the drug plasma concentration fluctuations at steady state during repeated dosing. Often this is expressed as a fluctuation index (FI)

$$FI = \frac{C_{\max} - C_{t=\tau}}{C_{ss}}$$

where C_{ss} is the average plasma drug concentration during the dosing interval.

$$C_{ss} = \frac{AUC_{\tau}}{\tau}$$

The variability in each primary variable is generally also an important factor to evaluate. Large variability for an MR formulation may give rise to dosing restrictions and bioequivalence may also be difficult to prove.

The most informative way of evaluating the MR formulation performance in vivo is to calculate the full-time course in vivo absorption- or dissolution-time profile and compare them with in vitro dissolution profiles. Usually, the absorption- or dissolution-time profile is calculated using a deconvolution method. In deconvolution, three different functions are defined; the input, weighting, and response functions. The input function corresponds to the entry of drug into the body, that is, the in vivo dissolution- or absorption-time profile of the tested MR formulation. The weighting function corresponds to the time course of the drug within the body, usually described by the drug plasma concentration-time profile after administration of an oral solution, or alternatively an IV bolus dose. Finally, the response function is the drug plasma concentration for the MR formulation. In deconvolution, the input function is calculated from the weighting and response functions. A review of different

methods for calculating in vivo absorption/dissolution-time profiles can be found elsewhere (73).

In Vivo Imaging

In vivo imaging technologies are important in the pharmaceutical development to visualize the GI transit and the drug delivery process. With these methods several factors relevant for the formulation function in vivo can be monitored directly. The GI transit variables comprise residence times or entering and emptying times in various parts of the GI tract. Factors more related to the drug delivery process are in vivo disintegration or erosion of solid formulations and distribution of multiple units in the GI tract. In vivo imaging studies are most useful when the imaging is combined with pharmacokinetic analysis of the drug plasma concentrations. In such combined studies the in vivo transit and drug release can be directly correlated with the drug absorption.

A number of noninvasive imaging technologies are available, but gamma scintigraphy has been described as the method of choice and is also the most commonly used imaging modality (74). In gamma scintigraphy, the drug formulation is radiolabeled using an appropriate radionuclide and, subsequently, visualized in the body by its emitted gamma radiation. With this method the obtained image is a two-dimensional representation of the body and the drug formulation distribution in the body. Several radionuclides are suitable for labeling drug formulations and factors such as radiation energy, half-life, and physicochemical properties of the available chemical form of the radionuclide need to be considered when choosing the most appropriate radionuclide. Metal ions, for instance ^{99m}Tc , ^{111}In , and ^{153}Sm , often meet the requirements on the radionuclide and hence are used extensively in scintigraphic studies. Because of the chemical nature of the radionuclide, the drug formulations are rarely labeled by chemical bonding of the radionuclide to the drug. Instead the radionuclide is generally incorporated in the drug formulation by mixing with the formulation components during manufacture.

Magnetic marker monitoring is an alternative imaging modality (75) with high spatial and temporal resolution in which no ionizing radiation is applied. This imaging modality relies on the in vivo monitoring of a magnetically labeled formulation using highly sensitive magnetic field sensors developed for bio-magnetic investigations. The labeling is achieved by incorporating a small amount of a ferromagnetic material, for instance the colorant black iron oxide, into the formulation and subsequent magnetization using a strong permanent magnetic field. From the recorded magnetic field of the labeled formulation, the three-dimensional localization and orientation of the formulation in the body are calculated. Furthermore, the strength of the magnetic source is obtained allowing for in vivo erosion or drug release studies.

Another potentially useful in vivo imaging technology is magnetic resonance imaging (76), but its use in pharmaceutical development has hitherto been limited. In this imaging modality, the magnetic resonance signal generated by nuclei with permanent magnetic moments (e.g., ^1H , ^{13}C , ^{19}F , and ^{31}P) when subjected to a strong magnetic field is utilized to construct two- or three-dimensional images. Although the signal from common nuclei is used, labeling of the drug formulation with a contrast agent may be required to enhance contrast. Other suggested imaging methods comprise PET, single photon emission computed

tomography, X ray, and ultrasound, but these methods seem to offer little or no improvements in the studies of GI transit and in vivo drug delivery.

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Immediate Release Oral Dosage Forms: Formulation Screening in the Pharmaceutical Industry

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INTRODUCTION

The goals of formulation development are to assure that the active pharmaceutical ingredient (API) is delivered in the clinic in a form that is acceptable to the patient and the clinician, is stable for the duration of intended use, and last but perhaps most importantly, provides adequate drug exposure at the site of action, which in turn results in the desired pharmacodynamic effect. During preclinical development, these goals have to be reached in predefined timelines to allow timely progression to clinical studies. Thus the development and use of models that can help predict the formulation performance is paramount to a successful formulation development process. From a biopharmaceutical standpoint, achieving adequate bioavailability is the key point of focus. The goal of this chapter is to provide an overview of the biopharmaceutical considerations during the formulation development process for immediate-release (IR) formulations delivered via the oral route in a pharmaceutical industry setting. Other important formulation screening components such as the evaluation of chemical/physical stability of the formulation and selection of an appropriate manufacturing process will not be the focus of this chapter.

Current State and Key Challenges in Pharmaceutical Formulation Development

Oral drug absorption consists of a series of processes that start with the disintegration of the dosage form, typically in the stomach, followed by dissolution, absorption and first-pass metabolism in the intestinal wall and/or the liver before the drug reaches the systemic circulation. The rate-limiting step in this cascade governs the oral bioavailability of the compound. A successful formulation ensures that the maximum amount of drug is available in an absorbable form, that is, in solution in the gastrointestinal (GI) fluids.

With the recent advances in combinatorial chemistry, biology and genetics, the number of drug candidates under development has been increasing. Because of the phospholipidic nature of cell membranes, a certain degree of lipophilicity is oftentimes a requirement for the drug candidate compound not only to be absorbed through the intestinal wall following oral administration but possibly also to exert its pharmacological action at its target tissue. While high lipophilicity is advantageous in terms of compound permeability, it intrinsically translates into poor aqueous solubility. Since the first step in the oral absorption process is dissolution of the drug compound in the GI lumen contents, poor aqueous solubility is rapidly becoming the leading hurdle for formulation scientists working on oral delivery of drug compounds (1).

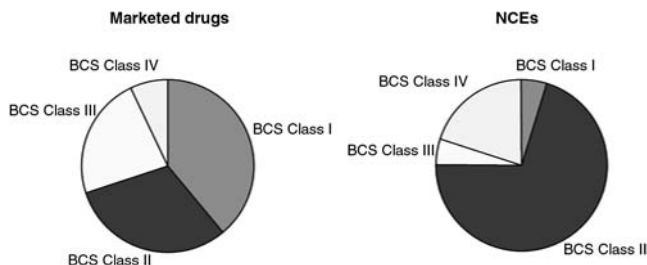


FIGURE 1 Biopharmaceutics classification system (BCS) class distribution of marketed drug compounds (*left*) and of new chemical entities (NCEs) currently under development (*right*). Source: From Refs. 4–6.

The trend toward poorly soluble compounds can be easily seen by looking at the biopharmaceutics classification system (BCS) classification of drugs currently on the market against the new chemical entities in pharmaceutical development. The BCS classification system, first proposed in 1995 (2) and introduced in 2000 as a bioequivalence tool by the Food and Drug Administration (3), categorizes the compounds in four classes based on their solubility and permeability, thus providing an easy categorization of the rate-limiting steps to absorption: BCS class I (high solubility, high permeability), BCS class II (low solubility, high permeability), BCS class III (high solubility, low permeability) and BCS class IV (low solubility, low permeability). A recent analysis of marketed compounds (Fig. 1), suggested that more than 50% of marketed drugs belong to the high solubility BCS classes I and III (4,5). In sharp contrast, the estimates for NCEs currently in development suggest that less than 10% of the compounds meet the high solubility criteria (6).

Poor aqueous solubility can lead to low and variable bioavailability. Low oral bioavailability can represent a significant challenge throughout the drug development process. It can pose a barrier to obtaining the necessary exposure margins in toxicology studies, cause deviations from the desired dose proportionality in the early phase I studies or necessitate the use of high doses to obtain the necessary exposures. High variability is equally not desirable, in terms of both ensuring consistent drug performance across different individuals as well as complicating any bioequivalence studies needed to bridge across formulations used at the different clinical stages. Finally, poor aqueous solubility is oftentimes associated with an undesirable food effect. A recent analysis conducted by Gu et al. (7) looking at a set of marketed compounds suggested that 71% of BCS class II and 73% of BCS class IV compounds were associated with a positive food effect, that is a significant increase in exposure following administration in the fed state (Fig. 2). All these hurdles frequently lead to utilization of specialized formulation technologies to overcome the inherent limitations of the compound's physicochemical properties and to deliver acceptable bioperformance in the clinic.

BCS Principles in Formulation Development

While the BCS has been established as a regulatory tool to facilitate formulation comparison and identify needs for clinical bioequivalence studies during filing (3), it is also widely adopted in the pharmaceutical industry throughout the

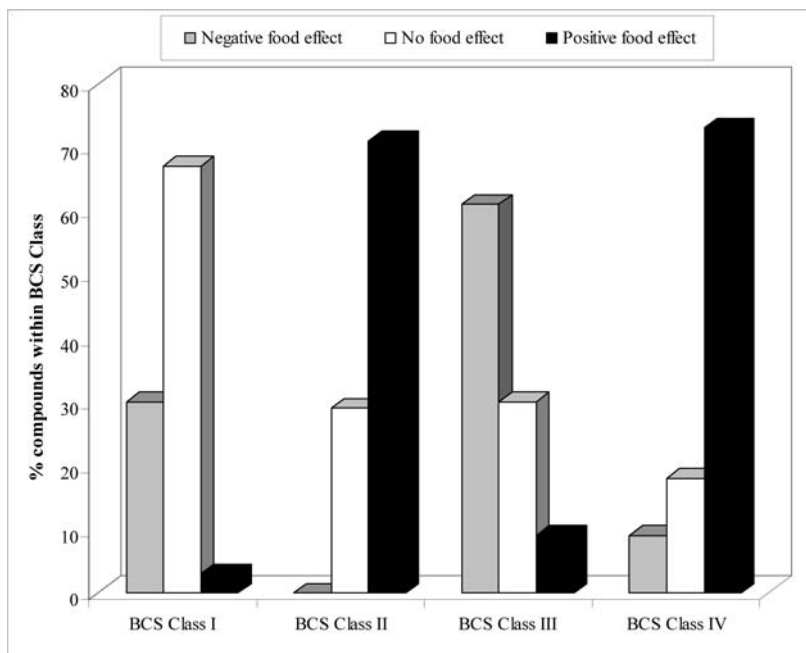
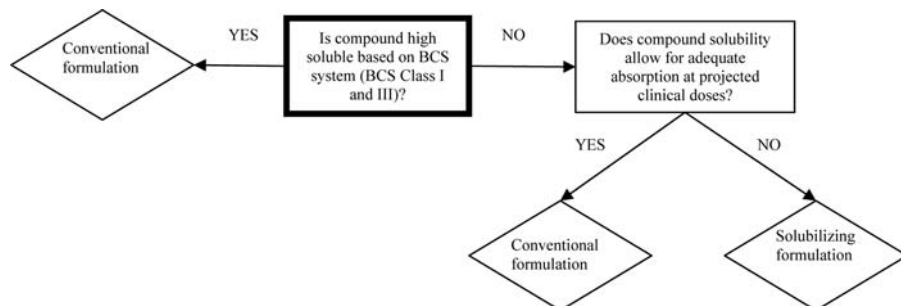


FIGURE 2 Prevalence of clinical food effect on total exposure as a function of biopharmaceutics classification system (BCS) class. *Source:* From Ref. 7.

drug development process from early stage assessment of developability of drug candidates to facilitating decisions on clinical bridging studies. Given the prominent role of solubilization as a driver for formulation decisions, it is perhaps not surprising that the BCS system is also used as a reference to guide formulation decisions. For example, a simple high-level decision depicted in Scheme 1 can provide a general direction for formulation scientists when deciding the type of formulation and process to explore, especially in the early development stage.

Formulation selection “decision trees” based on the BCS system and compound solubility have been published in the literature (8–10). A common



SCHEME 1 A general formulation development decision tree based on BCS-based solubility definition.

emerging theme from these decision trees is the use of low or high solubility category based on the BCS definition as the differentiating factor for selection of novel formulation technologies to increase in vivo solubilization. More specifically, for highly soluble drug candidates, conventional formulations (i.e., API granulated or blended with appropriate excipients to facilitate encapsulation or tableting) are typically pursued. If the solubility of the API does not represent a limiting factor to absorption, the role of the formulation is essentially to ensure fast disintegration once the dosage form comes in contact with the GI fluids. The effect of formulation on permeability is generally considered to be limited. Permeation enhancers (11) have been suggested as possible means to increase exposure of BCS class III compounds, however clinical evidence of their utility is by large lacking.

By contrast, decision trees for BCS class II and IV compounds are significantly more complex, opening the way for the use of solubilization technologies such as liquid-filled capsules (LFCs), solid dispersions or accelerating dissolution through nanosizing to achieve the desired bioavailability. For BCS class II and IV compounds, solubility is a major limiting factor to oral absorption. However it should be noted that despite their low solubilities, not all BCS class II and IV compounds require novel formulation technologies. Especially for BCS class II compounds, even though the solubility may not be sufficient to allow for solubilization in the standard 250 mL of water used in the BCS classification, rapid and continuous absorption of the compound in vivo can facilitate dissolution of larger amounts than would be indicated by the BCS classification. Thus, the typical approach is to develop metrics to quantify the extent of improvement in solubility needed and use those as the differentiating factor between conventional and solubilizing formulations. Such metrics can include a solubilization volume or a "dose number" cut-off value. For example, Ku (8) suggested the use of a solubilization volume of 5000 mL as the criterion for selection of a novel formulation technology. Since such a calculation only deals with a single parameter related to oral absorption, that is, the compound solubility, it may overlook other compound properties, such as permeability, that can play a compensatory role in the absorption process. Thus it would appear that for this approach to work effectively, the development of in-house databases for selection of cut-off values is necessary. An alternative to this approach is the use of absorption estimates such as the maximum absorbable dose (MAD) calculation [eq. (1)] proposed by Johnson et al. (12,13) or the absorption potential (AP) calculation [eq. (2)] proposed by Dressman et al. (14).

$$\text{MAD} = S \times K_a \times V \times \text{SITT} \quad (1)$$

$$\text{AP} = \log \left(P \times F_{\text{NON}} \times \frac{S_0 V_L}{X_0} \right) \quad (2)$$

where S is drug solubility, K_a is the permeation rate constant, V is the intestinal volume, SITT is the small intestinal transit time, P is the drug 1-octanol-water partition coefficient, F_{NON} is the nonionized fraction at pH 6.5, and X_0 is the drug dose.

The advantage of MAD is that it allows for a direct comparison of the desired clinical dose of the compound against an absorption estimate that can be compound specific. The downside is of course the need for additional data, including a relatively accurate estimate of the compound permeability.

Biorelevant Dissolution Testing in Formulation Screening

In vitro dissolution testing is an important aspect of the formulation development process as it enables the assessment of the drug release from the formulation. Traditionally, dissolution testing has been employed during pharmaceutical development as a quality control test to ensure performance of the formulation in respect to complete release of the intended dose. Such studies are typically run using compendial media [i.e., simple buffered systems with or without surfactants such as sodium lauryl sulfate (SLS) or Tween 80] and employ sink conditions to allow for 100% release of the API. These conditions aim to decouple any effects of the API solubility from the performance of the formulation itself.

However, during formulation development, it is becoming increasingly apparent that traditional dissolution methods may not be sufficient (15). Rather, biorelevant dissolution methods that allow for a prediction of the in vivo performance of the formulations are sought. A biorelevant dissolution method that provides an in vitro–in vivo relationship can allow the selection of the most suitable formulations to be tested in vivo, thus reducing the need of multiple animal studies, shortening the formulation development timeline and minimizing resource consumption. The need for biorelevant methods that take into account the solubility of the API in the dissolution medium, is especially important for the evaluation of solubilizing technologies where the role of the formulation is to produce a concentration of drug in vivo that exceeds its solubility. The ability to observe desired supersaturation phenomena is critical for formulation scientists to understand the potential benefits of unique formulations such as LFCs and solid dispersions over simpler conventional formulations. Traditional sink-condition dissolution methods may not provide sufficient discriminating power in that respect.

To better mimic the conditions a formulation meets in the GI tract, a series of biorelevant media have been proposed in the literature where physiologically relevant solubilizing agents such as sodium taurocholate and lecithin, replace the synthetic surfactants of the traditional test. Such media can be employed successfully to differentiate formulation behavior (16). More details on biorelevant dissolution methods and their application in formulation development are given later in this chapter as well as in chapters 12 and 13.

It is worth mentioning that the ultimate goal of in vitro dissolution is the establishment of an in vitro–in vivo correlation (IVIVC), that is, a mathematical relationship linking the dissolution results to one or multiple drug level descriptors (e.g., AUC, C_{max} , total pharmacokinetic profile). The field of IVIVC has attracted relatively low attention for IR formulations compared with the work done on controlled-release (CR) formulations. Limitations such as the really fast dissolution times frequently seen for the IR formulations are possible contributors to this status. However, IVIVCs may be feasible for poorly soluble compounds where the dissolution rate is the limiting factor to absorption. Furthermore, more complex models that take into account both solubility and permeability have been developed (17) and can be used to guide formulation development.

Animal Models in Preclinical Formulation Screening

Animal models are commonly employed in preclinical development to provide a basis for prediction of human pharmacokinetics as well to establish the safety and tolerability of the compound prior to administration in the clinic. Despite

the potential differences in physiological parameters between animals and humans, preclinical animal models such as dogs and nonhuman primates (NHPs) have proven successful in predicting relative performance of formulations in the clinic. Studies in the literature have demonstrated the utility of such models in predicting food effects, increases in bioavailability through solubilizing formulations, etc. Thus, animal pharmacokinetic studies are often employed to compare behavior of potential clinical formulations *in vivo*. Details on the use of animal models for formulation development in the pharmaceutical industry are given later in this chapter.

Absorption Modeling in Formulation Development

Parallel to advances in *in vitro* dissolution methodologies, another field that has been attracting attention as a tool to guide formulation development is the use of *in silico* models. The use of computational tools and molecular descriptors to predict compound physicochemical properties is a common practice during drug discovery to facilitate the selection of promising drug development candidates. The development of computational models that simulate the oral absorption process has enabled formulation scientists to link API or formulation properties such as API particle size and formulation release rate to the *in vivo* dissolution, which as discussed above dictates oral absorption for insoluble compound. Such absorption models can be further linked to systemic pharmacokinetic models, allowing for an *in silico* comparison of full pharmacokinetic profiles for different formulations. While custom-build code can be used for such purposes as demonstrated in some literature reports, the availability of more powerful commercial software packages greatly facilitates the use of absorption simulations during drug development. Such software packages include PK-Sim[®] from Bayer Technology Services, which is based on PB/PK modeling principles (18), the INTELLIPHARM[®] PKCR, which combines simulation of drug dissolution with a one- or two-compartment pharmacokinetic model, and the GastroPlus[™] software from Simulations Plus, Inc., which allows for simulation of oral absorption using a modification of the compartmental absorption and transit (CAT) model (19) (advanced CAT model) and subsequently links the oral absorption to systemic pharmacokinetics through standard compartmental or PB/PK modeling. Utilizing such models and taking into account all the relevant biopharmaceutical properties of the compound of interest such as permeability and solubility, one can *in silico* assess the potential advantage of changes in formulation or API properties in terms of improving oral bioavailability before proceeding to *in vivo* studies. Such computational models can be applied at different stages of formulation development process (20). Examples in the literature have demonstrated the utility of such tools in prediction of food effect (21,22), optimization of particle size (23,24) or even prediction of outcome of clinical biocomparison studies (25,26). Further details and case examples are given in chapter 16.

COMMON PROCESS FOR IMMEDIATE-RELEASE ORAL FORMULATION SCREENING AND EVALUATIONS

Depending on the physicochemical properties of the API and the type of formulation utilized, screening and evaluation of potential clinical formulations with regard to *in vivo* performance could range from a simple conventional

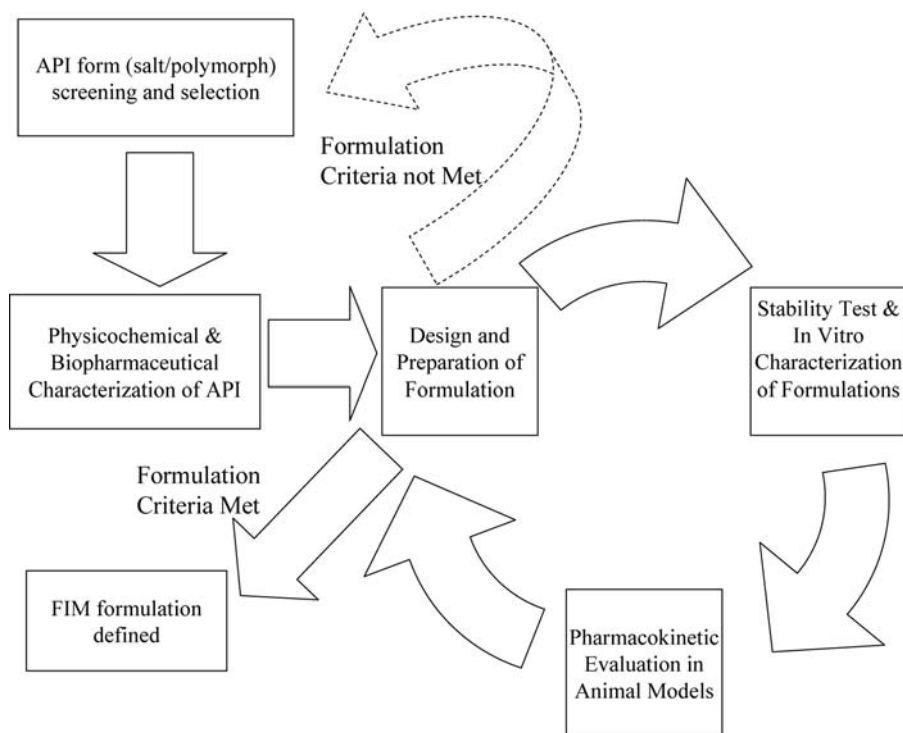
dissolution test (e.g., BCS class I and III compounds formulated in a simple solid dosage form) or a combination of complex labor-intensive *in vitro* tests and *in vivo* studies [e.g., BCS class II and IV compounds formulated with and/or without surfactant(s) in a polymer-based solid dosage form].

To rapidly develop a viable clinical formulation, several criteria have to be met including (i) physical and chemical stability, (ii) processability, and (iii) bioavailability. For formulations designed for early clinical studies, some flexibility on these criteria should be allowed to minimize the investment of resources on preclinical development candidates and to reduce the time to bring a candidate compound to the first-in-man (FIM) study. For instance, demonstrated short-term physical and chemical stability under restricted storage conditions (e.g., 5°C) for a given formulation can be acceptable to allow phase I single ascending and multiple dose studies because of the relative short duration of such studies. In addition, formulation processability, especially scalability, is normally not a major concern since phase I and phase IIa studies are typically conducted on a relatively small scale. While setting criteria for acceptable stability is relatively straightforward because of existing regulations and guidelines, qualifying the bioperformance of a clinical formulation could be a gray area, especially for early phase clinical usage. For most insoluble compounds, improving bioperformance often requires more time and resources for formulation development. In many cases, nonconventional and novel technologies (such as amorphous dispersions, LFCs, etc.) have to be employed. In an effort to obtain a balance between short development timelines and adequate formulation bioperformance, many companies have established road maps or decision trees in recent years to streamline early clinical formulation development. Simple solution or suspension formulations and capsules filled with neat API powders have been employed in early clinical trials. While development of these simple formulations consumes minimal resources, the potential downside is poor or suboptimal bioperformance, which in turn could lead to inability to achieve adequate exposures in early clinical evaluations. Therefore, caution should be taken when employing this approach, so that premature termination of a drug candidate or unnecessary delays in clinical studies caused by the need to develop alternative formulations can be avoided.

Obviously for late-phase clinical formulation development (e.g., phase IIb and beyond), stability and processability as well as the bioperformance aspects must be taken into consideration. Requirements for physical and chemical stability have to be met to ensure that the specified shelf life can be achieved. In addition to scalability, manufacturing dosage forms in large quantities also requires a comprehensive evaluation of other factors such as cost of goods, process robustness and, if applicable, intellectual property.

Typical Work Flow in Developing Clinical Formulations

Development of clinical formulations requires the participation and collaboration of many technical areas in a typical pharmaceutical company. While each company organizes itself in a unique structure, the core formulation development activities (Scheme 2) often take place in Pharmaceutical Research and Development (Pharm R&D). Regardless of the company organization scheme, as many as five types of activities are typically required to enable the transformation of an API to a viable clinical dosage form. As illustrated in Scheme 2, to maximize the efficiency in formulation development, the five technical areas are

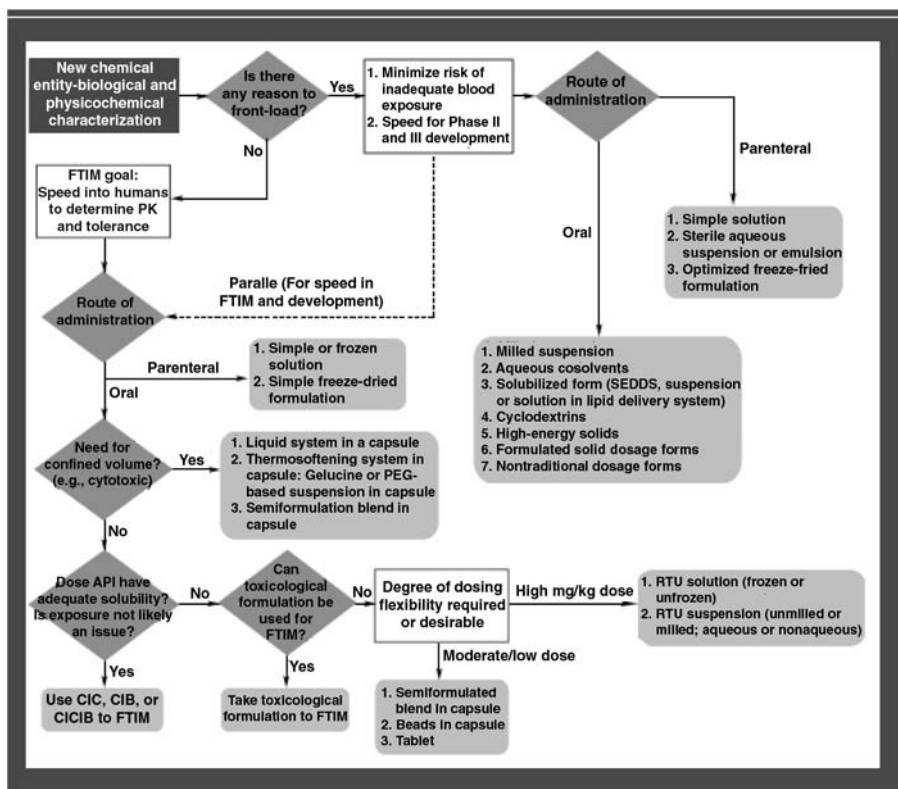


SCHEME 2 Typical activities conducted in early clinical formulation development.

typically integrated with close interactions. The API physicochemical and biopharmaceutical properties (e.g., pH-solubility profiles, BCS classification, physical and chemical stability of the selected API forms, API compatibility with common pharmaceutical excipients) often serve as the foundation for designing a clinical formulation. Prototype formulations are prepared and further screened by *in vitro* and/or *in vivo* methods to establish stability information and bioperformance. Depending on the complexity of formulation development (e.g., poor stability, high dose number, poor exposure in preclinical *in vivo* models), development teams can cycle through this process a few times until a viable formulation is identified.

Common Strategies for Developing Clinical Formulations

The continued low probability of success to reach late-phase development and the increasing cost for developing a new drug have prompted many companies to rethink the strategies for developing clinical formulations. In the last several years, many companies have adapted a new strategy in which a large number of compounds are entered into preclinical development, in the hope that a net increase in product registrations can be achieved. To accommodate the significant increase in preclinical development pipeline without the proportional increase in resources, the new focus has been on the reduction of cycle time and resources to develop and manufacture FIM formulations. Depending on the

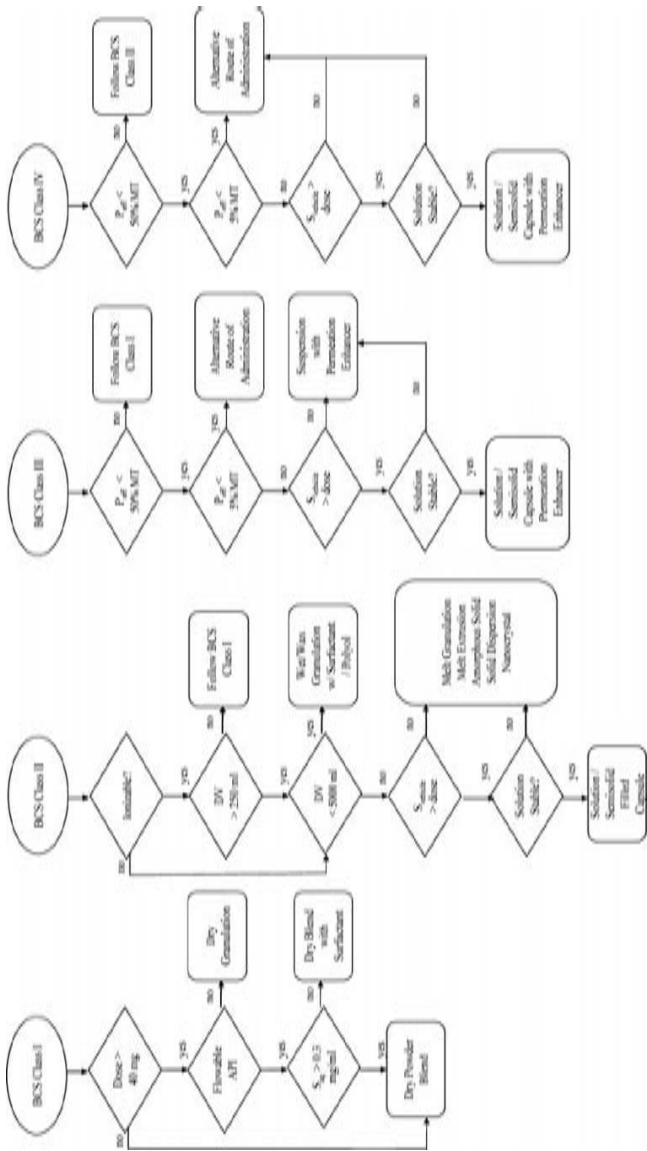


SCHEME 3 First-in-man (FIM) formulation decision tree. *Source:* From Ref. 9.

overall characteristics, a FIM formulation could be used for both phase I single and multiple dose studies as well as phase IIa proof-of-concept (POC) studies.

An early decision on the need for investment of resources in the preclinical phase can greatly facilitate decisions around FIM formulation development. An example of such a decision tree around meeting needs for FIM studies is shown in Scheme 3 (9). If the primary goal is to reduce the formulation effort required to dose a compound in humans to determine pharmacokinetic parameters and tolerability and to allow the rapid screening of multiple potential drug candidates, simple formulation approaches such as chemical-in-bottle (CIB) for reconstitution or resuspension, chemical-in-capsule (CIC), ready-to-use (RTU) solution/suspension, and granules or beads in capsules can be considered as potential options. The formulation used for toxicology studies can also be considered, if excipient levels are suitable for dosing in the clinic. On the other hand, when investment of resources is needed preclinically, additional steps should be taken to ensure optimal bioavailability, such as use of milled API, use of cosolvents or solubilizing agents, etc.

An alternative approach to formulation development undertaken by some pharmaceutical companies is to tie formulation decisions to the BCS classification of the compound, such as the decision tree reported by Ku (8). Scheme 4 captures the author's proposed decision trees for fast development of FIM



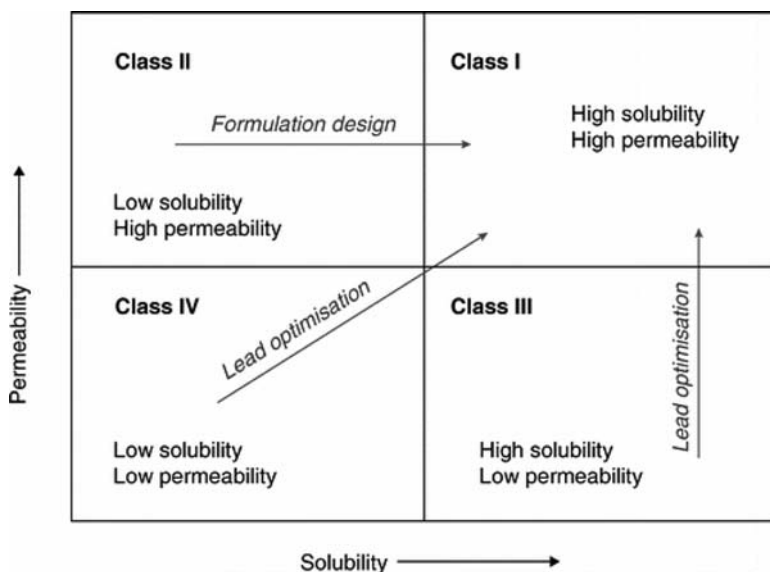
SCHEME 4 Example of first-in-man formulation development flow charts based on biopharmaceutics classification system (BCS) classification. Source: From Ref. 8.

formulations for compounds in each BCS class. For example, for a nonionizable BCS class II compound with a dose volume (DV) no more than 5000 mL, a traditional wet-granulation with surfactants or wax-granulation with polyol would be regarded as a potentially effective solution for an orally bioavailable FIM formulation. In contrast, for a nonionizable BCS class II compound with a DV greater than 5000 mL, lipid-based or solid dispersion formulations using hot-melt extrusion or spray-drying technologies may be required to achieve adequate exposure in humans.

While decision trees based on BCS classification primarily focus on API solubility to select a conventional or solubilizing formulation technology, efforts to use a wider set of molecular descriptors such as dissociation constants, molecular size, hydrogen bond donors and acceptors, etc., to guide formulation development have also been proposed. In one such example, Branchu et al. (27) reported the use of such descriptors along with statistical analysis to allow not only for selection of a conventional or nonconventional formulation but to further allow a differentiation between liquid formulations, solid dispersions and nanonization. Such statistical tools, provided sufficiently large data sets for their development exist, may allow more focused selection of clinical formulations right from the early stages of drug development.

As discussed earlier in this chapter, the prevalent application of combinatorial chemistry and/or biologically based high-throughput screening in drug discovery has led to an increasing number of poorly water-soluble compounds in pharmaceutical development pipeline. This has necessitated the need to employ novel drug delivery strategies to allow administration of such compounds by either the oral or parenteral route. An appropriate formulation can in practice switch the BCS classification of a compound by providing solubilizing capacities far beyond the compound's aqueous solubility. However, while formulation can greatly enhance the dissolution/solubilization process, it generally has a limited effect on compound permeability. This is why some pharmaceutical companies opt to address issues such as poor permeability at earlier stages of drug discovery, such as lead optimization prior to selection of drug development candidates (28) (Scheme 5). Consideration of biopharmaceutical issues at that stage can allow for modification of compound structure to build the necessary biopharmaceutical properties in the molecular structure.

It is worth mentioning that the trend to acceleration of FIM formulation development by simplification of formulations has introduced a new challenge for bridging formulations in the clinic (assuming that the development compound successfully passes the initial stages of clinical trials). Compared with the traditional commercial formulation development approach, the exploratory formulation development approach employs simple solution/suspension formulations or nonoptimized solid dosage forms, which are not viable for late-phase clinical studies and commercial usage. Changing a phase I solution formulation containing a large volume of cosolvent to a solid dosage form containing stable crystalline API often leads to changes in both rate and extent of absorption. Similarly, switching from a well-dispersed suspension formulation to a solid dosage form can also result in changes in pharmacokinetic parameters. Therefore, a formulation switching strategy, based on a good understanding of the physicochemical and biopharmaceutical properties of the API, formulation composition and process, and desirable pharmacokinetic characteristics for the target indications, should be considered when designing FIM formulations.



SCHEME 5 BCS of drugs. The absorption of BCS class II drugs can be markedly enhanced by optimal formulation design, whereas the best solution to improve the bioavailability of BCS class IV compounds is to go back to the lead optimization phase of drug discovery and modify their structures for the appropriate physicochemical properties. Abbreviation: BCS, biopharmaceutics classification system. *Source:* From Ref. 28.

IN VITRO SCREENING OF IMMEDIATE-RELEASE ORAL FORMULATIONS

In vitro dissolution assays have been traditionally employed as a quality control tool. The focus of such assays has been to demonstrating full release of the API from the dosage form. Release of the API within a prespecified time-frame has more recently been used as a surrogate for clinical performance in certain cases (e.g., IR formulations which can release 85% of the API within 30 minutes are considered fast dissolving in terms of biowaivers based on the BCS system).

With the increasing complexity of formulations required, especially for new drug candidates that are poorly soluble, it has become apparent that an even earlier introduction of in vitro characterization tools in the drug and formulation development process is needed. Appropriate in vitro tools that can serve as the first line of screening for formulations can lead to a significant reduction in the timelines required for preclinical formulation development. Not only can they reduce the resources required for parallel evaluation of formulation technologies but also minimize resources and time required for screening formulations in preclinical animal models.

Importance of In Vitro Characterization

The goal of in vitro characterization during the formulation development process is to maximize the possibility that dosage forms selected for further development will provide adequate bioavailability in the clinic. While in vivo

studies in preclinical species are also employed in this stage, because of their time- and resource-consuming nature, *in vitro* screens are preferred to allow for fast formulation screening and decision-making.

The scope of *in vitro* characterization in the formulation development process can vary significantly depending on the stage of development (e.g., prior to or after clinical data are available), the type of formulations being evaluated as well as the level of accuracy required for prediction of *in vivo* performance. *In vitro* characterization usually starts as early as the identification of a suitable drug candidate. At that stage, measurement of basic physicochemical properties such as solubility in buffers and biorelevant media can provide a general guidance to selection of formulation technologies (see section "Common Strategies for Developing Clinical Formulations") and identify potential clinical issues (e.g., a food effect would be indicated by significant solubility differences in media simulating the fed and fasted state) that could be addressed through specialized formulation efforts.

Subsequently, during early stages of formulation development, prior to introduction of the compound in the clinic, the role of *in vitro* screening is largely to help rank order formulation candidates in terms of achieving adequate solubilization. More often than not, the *in vitro* tests at this stage are supplemented by *in vivo* studies in preclinical species with the lead formulation(s). Given the diversity of formulation options, *in vitro* screens are used to provide answers to a variety of questions. For conventional formulations such as roller-compacted (RC) or wet-granulated (WG) tablets, identification of a need for and selection of surfactants oftentimes relies on *in vitro* wetting and dissolution tests. Similarly, for compounds that are expected to precipitate *in vivo* (e.g., salts of weak acids following initial dissolution in the stomach), screening of antinucleating agents can be accomplished via *in vitro* methods. When such conventional dosage forms fail to achieve the desired bioavailability, *in vitro* screens are utilized to measure the degree of enhancement in solubilization achieved through novel technologies. For LFCs where the drug is introduced in solution form to the body, *in vitro* tests can be used to assess the potential for *in vivo* precipitation upon dilution of the capsule fill in the GI fluids. For formulations that rely on the enhanced solubility of the amorphous form of the API, *in vitro* screening can be used to measure the degree of supersaturation obtained over the crystalline API form. In other cases, the increase in the dissolution rate is more important, for example, when examining the effect of reduction of particle size on bioperformance either for conventional dosage forms or to identify the need for use of nanosized API. Finally, *in vitro* dissolution can be used to estimate the extent of food effects for the selected formulations, either to guide formulation technology selection or help with the design of clinical studies.

Once clinical data is available, the scope of *in vitro* dissolution assays oftentimes shifts from providing differentiation among formulation candidates to becoming a more predictive tool of clinical bioperformance. The development of "biorelevant" dissolution methods at this stage can help with further optimization of the clinical formulation and may involve the establishment of IVIVR or IVIVC in a more quantitative fashion than is possible in early development. The establishment of an IVIVC can prove extremely beneficial during the late stages of drug development, especially if it enables a simple *in vitro* test to be used as a surrogate for clinical bioequivalence studies.

As already mentioned, the importance of *in vitro* characterization in this stage is derived from the potential significant savings it offers in terms of time and resources in the formulation development process. *In vitro* screening tools, especially with the increase in automation in the recent years, enable rapid screening of multiple formulation candidates while minimizing consumption of API. Early identification of lead formulation and technologies eliminates the need for parallel development and allows for focused utilization of resources. Biorelevant dissolution methods serve as surrogate of time-consuming studies in preclinical species, further speeding up the formulation development process and increasing confidence in decisions around formulation bridging. Given these potential benefits, it is not surprising that the field of biorelevant dissolution has been gaining increased attention in the recent years from both pharmaceutical industry and regulatory agencies.

It is worth mentioning that the traditional role of dissolution as a quality control test remains an integral part of the formulation development process. Dissolution tests to ensure release of API meets the required specifications are typically conducted on any final clinical formulation before the supplies are deemed suitable for clinical use. However, since the focus of this chapter is on formulation development process related to selection of formulation technologies, the quality control aspects of dissolution testing will not be covered further in this chapter.

Common In Vitro Methods

In vitro characterization tools related to bioperformance of the dosage form encompass a large variety of assays that can help with decisions around formulation optimization. They range from the use of robots to obtain solubility measurements in a high-throughput fashion all the way to the traditional dissolution testing. Some of the most common *in vitro* tools used in the pharmaceutical industry are described below.

In vitro characterization starts early with measurements on the API itself. Utilization of automation robots enables rapid generation of solubility data. *In vitro* tests such as measurement of contact angle or flotation tests can be used to measure the wettability of compounds, and thus identify the need for the use of surfactant in the formulations. Solubility measurements at different surfactant levels provide more data toward this decision. Screening of antinucleating agents can be accomplished by measuring the formation of particles in the media of interest. As far as dissolution tests are concerned, these can be categorized into two major groups: sink-condition dissolution tests and biorelevant media dissolution tests.

To achieve sink conditions, dissolution tests are conducted under conditions in which the solubility of the API in the media is adjusted to exceed its solubility at the corresponding pH (oftentimes by a factor of three or more) with the use of surfactants. By contrast, dissolution tests with biorelevant media are performed under conditions where the media composition is fixed to represent the contents of the GI tract and is not adjusted to account for the possibly poor solubility of the tested compound in the medium. Dissolution tests under sink conditions encompass the vast majority of quality control tests. Media used in such tests usually consist of buffer systems with or without surfactants. For example, a simple 50 mM phosphate buffer at pH 6.8 (29) is used for atorvastatin calcium because of the favorable solubility of this

acidic compound at pH 6.8. By contrast, 0.5% SDS is added to an aqueous buffer (29) to generate sink conditions for the dissolution of simvastatin in its lactone form. The goal of such tests, as mentioned already, is typically to capture complete release of the dose from the dosage form. However, dissolution tests with such compendial media might be considered biorelevant in cases of weak acids, where solubility in the intestinal tract oftentimes allows for full solubilization of the dose, and for weak bases, where initial solubilization takes place in the favorable acidic environment of the stomach. Sink conditions may also help identify differences in initial dissolution rates between formulations that are related to dosage form behavior (e.g., disintegration vs. erosion) or to provide better prediction of clinical performance (compared with nonsink, biorelevant conditions) for highly permeable compounds, where the absorption process actually drives the *in vivo* dissolution by creating sink conditions *in vivo*. Finally, for IVIVC purposes, the compendial media offer significant flexibility to the scientist to adjust the *in vitro* dissolution rate to correspond with *in vivo* results.

Nonsink dissolution involves the use of the so-called “biorelevant” media, since they are prepared in a way to match the contents of the GI fluids. Recipes for biorelevant media can be found in the literature by numerous groups. The human fasted-state small intestinal fluid (FaSSIF) and fed state-simulated intestinal fluid (FeSSIF), initially proposed by Dressman et al. (30), are two of the most commonly used media to simulate the intestinal contents, while simulated gastric fluid (SGF) is typically used as a surrogate of dissolution in the gastric fluid.

While the parallel permeation and dissolution processes dictate the extent of oral absorption *in vivo*, mimicking the contents of the GI tract may be more important than ensuring sink conditions, if the goal of the dissolution is to facilitate formulation development. As a result, these biorelevant media have been successfully employed in screening formulations both within as well as across different technologies. Since the solubility of the API in the media is not artificially increased through the use of surfactants, these media also allow for the observation of any supersaturation.

In terms of apparatus used for the dissolution tests, USP apparatus II (paddle) and USP apparatus I (basket) appear to be the first choice for *in vitro* dissolution testing of IR dosage forms, particularly solid formulations. While USP apparatus I and II enable testing of a single dissolution medium, the flow-through cell USP apparatus IV can be used to change media and/or flow rates during the dissolution test, as well as offering the flexibility of operation in an open- (continuous supply of new media) or closed-loop (recirculating) mode. Thus USP apparatus IV may prove useful in cases where changing the pH of dissolution is desired. In conjunction with the use of specialized flow-through cells, the USP IV apparatus has also been proposed as being the most suitable for the dissolution testing of LFCs (31).

Depending on the dosage form used, different *in vitro* tests may be applicable. While dissolution tests as the ones described above are frequently employed for solid dosage forms, for LFCs they may be of limited value. Serial dilution experiments to test for precipitation or redispersibility experiments where the size of droplets upon dispersion is used as a surrogate for *in vivo* performance may be more informative (29). Depending on the type of the formulation, studies where digestion of the formulation components (typically

fatty acids) is taken into account may provide an even more accurate view of in vivo behavior (32,33).

In Vitro Screening of Clinical Formulations

In vitro assays should be the first line of screening to help with formulation definition. As a result, in the early stages, the focus of dissolution tests is on selection of excipients that will facilitate the dissolution of the API from the formulation. For conventional formulations of BCS class II and IV drugs, this often entails the selection of surfactants that will help with wetting and possibly solubilization of the poorly water-soluble API.

Studies to address the wettability issue are typically performed initially using the pure API and solutions of different surfactant concentration to help narrow the possible formulation options. At this stage visual observation of wetting or measurement of the contact angle may be sufficient. Lead excipients are subsequently incorporated in the formulation, and the dissolution test is conducted again to assess enhancement of the dissolution rate. This procedure can be illustrated with a Merck drug development candidate. Compound A is a weak base, dosed as the hydrochloride salt, and having a high solubility at gastric pH (>15 mg/mL at pH 1–3) but low solubility in the intestinal pH range (10 µg/mL at pH 7.5). On the basis of Caco-2 permeability data, compound A has been classified as a BCS class II compound. Assuming that dissolved drug emptying from the stomach quickly permeates the intestinal wall (without precipitating), fast dissolution in the stomach is needed to maximize bioperformance. Despite the high solubility of compound A at gastric pH, poor wetting was observed as indicated by the slow and incomplete dissolution of the API in SGF with less than 60% of the API dissolved in the first 30 minutes, which can be taken as an estimate of the gastric residence time in the fasted state. Addition of a surfactant, in this case poloxamer 407 significantly improved the dissolution rate and approximately 80% of the API was dissolved within the first 10 minutes of the dissolution (Fig. 3). Since the specific screen was conducted using a high dose estimate to cover the phase I study range, dissolution of the poloxamer-based formulation was expected to be even more favorable at the lower potencies. On the basis of the results of the screen, the poloxamer-based formulation was adopted as the lead formulation for further development and clinical use.

Screening of excipients for formulations does not always rely on dissolution assays. In the case of antinucleating or dispersing agents, for example, the desired outcome from their incorporation in the formulation is to prevent precipitation as large particles that would prove problematic for dissolution. Here, measurements of precipitation in vitro may be sufficient to guide excipient selection. A case example is the Merck drug development candidate, compound B (34,35). Compound B is the potassium salt of a weak acid and was classified as a BCS class II compound. The free acid form of compound B was practically insoluble in the stomach fluid with a solubility of less than 7 µg/mL. Maintaining the dissolved salt in solution in the stomach (or at least minimizing the particle agglomeration and particle size of the suspension formed by precipitation by gastric acid) would therefore be beneficial in terms of maximizing bioavailability. In this case, the in vitro screening was focused on measuring the resulting particle size distributions following addition of API-surfactant solutions to 0.05 N HCl. As seen in Figure 4, incorporation of poloxamer 407 resulted in smaller particle size with a narrower distribution upon

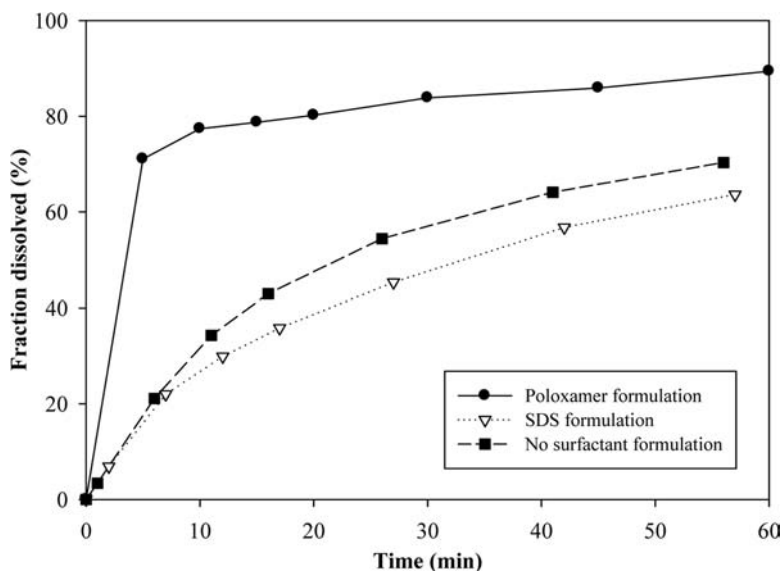


FIGURE 3 Effect of surfactant on dissolution of a weak base drug development candidate. Dissolution was conducted in 250 mL of simulated gastric fluid medium (USP II, 50 rpm). A clear effect of surfactant (poloxamer 407) in facilitating dissolution of the active pharmaceutical ingredient was observed.

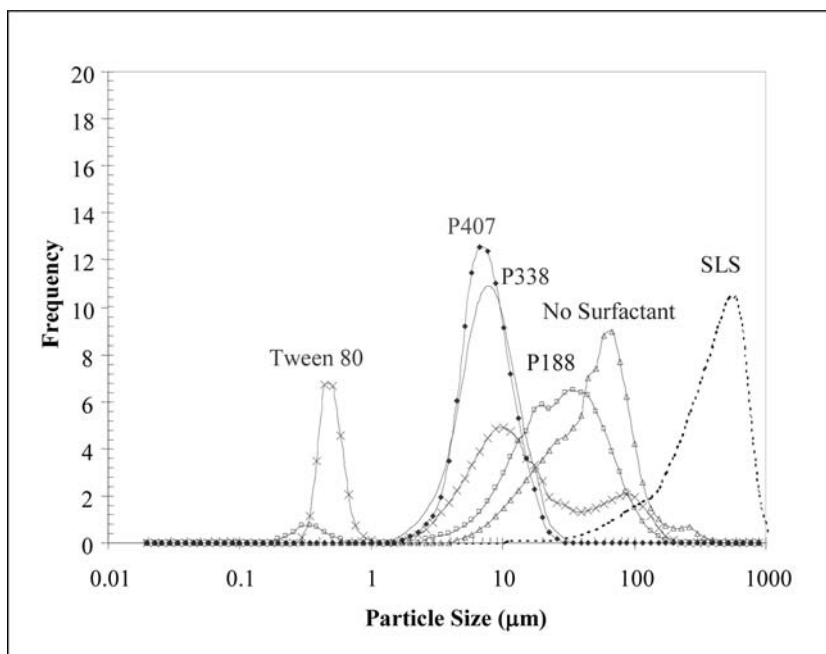


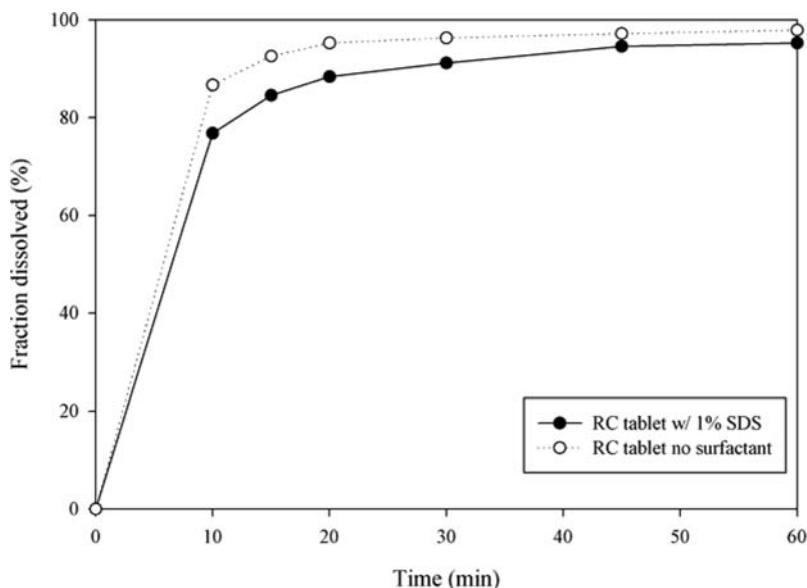
FIGURE 4 Particle size distribution for suspensions derived from mixing compound B in water with 0.05 N HCl solution containing one of specified surfactants.

redispersion, compared with the addition of SLS, a lower-molecular weight poloxamer 188 or no addition of surfactant. It should be noted that the formation of stable and fine suspensions by poloxamer 407 and Tween 80 was easily visualized compared with other mixtures. On the basis of the data from such a screen, formulation development can proceed by testing formulations in either further *in vitro* dissolution screens or *in vivo* studies in preclinical models (see section "Animal Screening of Oral Clinical Formulations").

Data such as those generated above can also trigger the employment of novel formulation technologies. Since particle size of the resulting suspension could be modulated through the use of suitable excipients, one could envision nanosizing the API to provide even better control of the particle size upon redispersion of the formulation in gastric juice. In fact, for compound B, a stable nanosuspension with an average particle size of 200 nm was prepared using the ELAN nanomilling technology and was shown to significantly improve exposures over micronized API in preliminary screening in rodents.

While the case studies presented so far demonstrate the use of such tools to select excipients, dissolution assays can be utilized similarly to justify removal of an excipient from the formulation. As discussed above, one of the early tests to assess the need for incorporation of surfactants is the wettability assessment. However, while a surfactant may significantly improve wetting in *in vitro* tests, inclusion of a surfactant in a formulation may not always translate to significant advantages in *in vivo* dissolution. One such case can be demonstrated by a Merck drug development candidate. Compound C is a BCS class II weak acid dosed as the free acid form. Solubility in biorelevant media (FaSSIF) was deemed adequate to solubilize the intended clinical dose. On the basis of wettability experiments, the initial FIM formulation was prepared by incorporating SLS in the formulation and was shown to provide good bioavailability in preclinical models and subsequently in humans. As the compound progressed in clinical development, the question as to whether SLS was crucial to this formulation was raised because of concerns about scale-up of the process. A dissolution experiment was conducted in which the intended clinical image was tested, with and without surfactant, in a dissolution vessel containing 500 mL of FaSSIF. As seen in Figure 5, no significant differences were observed in either the rate or extent of dissolution. The results from the *in vitro* test were subsequently verified in an *in vivo* study in dogs where formulations with and without SLS showed comparable exposures. The formulation without surfactant was chosen for use in subsequent clinical trials.

With the increase in the number of insoluble compounds in development, a major goal of *in vitro* screening is the ability to differentiate between novel solubilizing and conventional formulations. In the case of nanosuspensions, for example, the increase in dissolution rate is the driving force behind the improvements in bioavailability oftentimes seen in the clinic. In the case of amorphous dispersions, attainment of supersaturation is sought. One example of evaluation of such a new technology *in vitro* was published by Hecq et al. (36). In that study, Hecq and coworkers studied the dissolution behavior of nanosized API of the poorly soluble weak base ucb-35440-3 at different pHs between 3 and 6.5. The dissolution experiments at all pH values clearly indicated that the nanosuspension results in a much faster and also more complete dissolution compared with micronized API. Such dissolution tests can be used very early on to guide formulation development toward these new technologies.



Formulation	AUC _{0-24 hr} ($\mu\text{M}\cdot\text{hr}$)	C _{max} (μM)	T _{max} (hr)
RC tablet w/1% SDS	797 $\pm$ 81.7	69.4 $\pm$ 9.8	2.7 $\pm$ 0.7
RC tablet w/o SDS	872 $\pm$ 44.9	77.1 $\pm$ 5.5	2.6 $\pm$ 0.9

FIGURE 5 Dissolution of compound C formulations in FaSSIF (500 mL, USP II, 75 rpm) suggested no significant advantage from incorporation of surfactant. The observation was opposite to initial data on active pharmaceutical ingredient wettability. Subsequent study in dogs confirmed the results of the dissolution test.

When LFCs are employed as formulations for poorly water-soluble compounds, traditional dissolution tests may not be appropriate to compare bioperformance of formulations. For LFCs with a vehicle (e.g., a lipid and surfactant combination) that forms an emulsion upon dispersion in the GI fluids, assessment of the emulsification capacity may be sufficient to rank order formulation bioperformance. One of the most commonly cited examples in the literature is that of cyclosporine Neoral and Sandimmune formulations. The original formulation of CsA, Sandimmune[®], was an oil-based emulsifying formulation that formed micron-sized emulsions (mean droplet size of 3.7 μm). However, a significant food effect (37%) was still observed. Neoral was developed to increase bioavailability of CsA by reducing the droplet size of the resulting emulsion to below 100 nm (37,38). Later, it was shown that the droplet particle size after dispersion can be linked to measurement of compound in solution in the medium, providing an additional level of quantification of solubilization capacity (39). As a result of increased bioavailability of CsA, Neoral exhibited a minimal food effect.

Dissolution testing can also be used in mid to late-stage formulation development to address specific clinical needs. For example, for weak bases,

depending on the intended population of administration, interactions with antacids can be of a concern. An increase in stomach pH can prove detrimental for the bioavailability of weak bases with pK_a values in the 2-to-5 region, where dissolution in the acidic stomach is generally required for optimal bio-performance. In such cases, novel formulation strategies may need to be employed to minimize the dependency of dissolution on gastric pH. Badawy et al. (40) reported the use of dissolution assays to address this question for formulations of BMS-561389, a weak base with solubility of ~ 2 mg/mL at $pH < 3$ but only 0.2 $\mu\text{g/mL}$ at $pH > 6.5$. The authors prepared a series of formulations incorporating different acidic excipients to help with solubilization of the API. It was demonstrated through in vitro dissolution at the pH of 5.5 that incorporation of tartaric acid could facilitate initial dissolution of the API, providing more than a 2-fold increase in the total amount dissolved under this dissolution condition compared with a control formulation with no acidic excipients. Favorable precipitation kinetics in this case ensured bioavailability of the new formulation regardless of administration in normal or elevated gastric pH conditions. The results of the dissolution studies were confirmed in vivo (see section "Animal Screening of Oral Clinical Formulations").

Another common clinical issue that could be addressed through formulation efforts is the positive food effect frequently encountered for BCS class II and IV compounds. Positive food effects are usually associated with significant increases in solubility of the compound in the GI lumen after a meal, both because of the fat present in the food as well as the increased secretion of endogenous bile acids that further solubilize the drug. Food effect screening in vitro can be conducted using biorelevant media to simulate the fasted (FaSSIF) and fed (FeSSIF) states. Formulations that significantly increase the dissolution rate in FaSSIF can be considered as food effect mitigating formulations. One such example is provided by Merck development compound D. Compound D is a weak base with poor solubility in the intestinal pH range and was categorized as BCS class IV on the basis of Caco-2 permeability data. A conventional formulation (formulation A) was initially developed but showed a significant food effect. To address the food effect, formulations based on solubilization technologies were subsequently screened in vitro to ascertain the increase in rate and extent of dissolution. As seen in Figure 6, formulation B, a solid dispersion formulation, significantly increased the overall solubilization of compound D in FaSSIF medium. In subsequent preclinical screening using a food effect model in dogs, formulation B was shown to eliminate the substantial food effect that was observed for formulation A.

Finally, at late stages of formulation development, dissolution assays are intended to ensure bioequivalence of formulations to facilitate any bridging needed in the clinic. Similarity in dissolution can be judged by applying the f_2 similarity criterion if the dissolution method is considered predictive of formulation behavior in the clinic (but may not necessarily utilize biorelevant media such as FaSSIF), following similar criteria as those outlined in the SUPAC guidance. In the case of BCS class I compounds, such dissolution results may be used as supportive data for biowaivers. It should be noted that the f_2 factor would be suitable as similarity criteria in cases where 100% dissolution is achieved. However this may not be achievable for BCS class II and IV compounds in biorelevant media such as FaSSIF. In those cases, if alternate media that satisfy the requirement for complete release as well as for bioperformance predictability

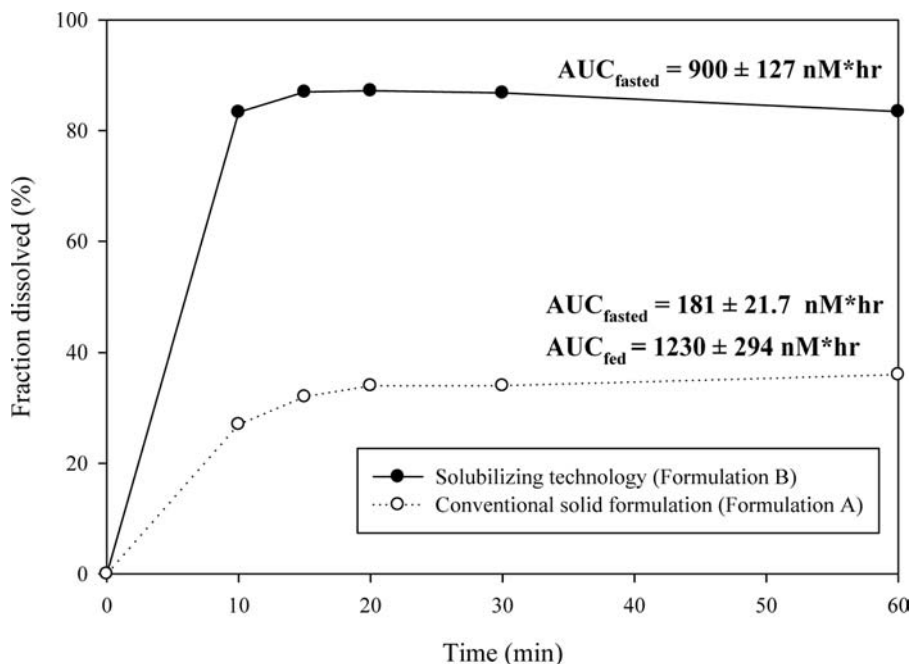


FIGURE 6 Utilization of biorelevant dissolution (500 mL, USP II, 100 rpm) to identify food effect-mitigating formulation. The dissolution of conventional formulation A, which exhibited a significant food effect, was compared in FaSSiF with that of the solubilizing formulation B. A significant increase in fraction dissolved was observed for the latter. The enhanced solubilization capacity was also confirmed in subsequent *in vivo* studies in dogs (AUC values indicated above the corresponding dissolution curves).

cannot be identified, leveraging prior knowledge on the IVIVR (in vitro–in vivo relationship) between dissolution in the biorelevant media and bioavailability can help assess the risk of switchability between formulations.

Challenges and Limitations of In Vitro Models

As detailed in section “In Vitro Screening of Clinical Formulations,” *in vitro* tools can play an important role in guiding formulation selection. However, while *in vitro* characterization tools have been proven successful in several cases, obtaining truly predictive dissolution data is often a challenge faced by researchers during formulation development. Questions around biorelevance of *in vitro* data frequently emerge during the early steps of formulation development, when *in vivo* data are not available. While obtaining *in vivo* data can help with adjusting the dissolution conditions to achieve the desired IVIVR, it is not uncommon to encounter a situation where correlation between the *in vitro* data and the *in vivo* outcome is very limited.

The challenges around *in vitro* tools commonly employed in formulation development process perhaps should not come as a surprise, considering the complexity of the *in vivo* dissolution/absorption process against what can be achieved using current *in vitro* techniques. The transition of the API/formulation

from the stomach to the intestine, the simultaneous absorption process that can provide a driving force for further solubilization, the potential *in vivo* effect of bile salts and digestive enzymes (perhaps more important in the case of LFCs) and the *in vivo* hydrodynamics as related to water absorption/secretion are all complex processes that are difficult to capture with *in vitro* experiments or without introducing significantly complex and time-consuming experimental setups that negate the sought after high-throughput capability of the *in vitro* assays. While “biorelevant” media such as FaSSiF have been described in the literature and are commonly employed, discrepancies between the composition of such media and that of *in vivo* fluids have been reported (21,41,42). For a large percentage of the formulations, dissolution in the stomach will largely dictate their bioperformance; however a media that accurately represents the *in vivo* solubilization capacity of gastric fluid remains elusive. The use of *in vivo* aspirates has been proposed as a solution to these shortcomings of artificial media, but the practicality of this approach for routine formulation screening remains to be proven. In addition to the physiological factors described, the increasing diversity of formulation technologies employed adds an additional layer of complexity to the appropriateness of *in vitro* characterization tools. For example, in the worst case scenario where multiple formulation approaches (lipid-based liquid formulation, polymer-based solid dispersion, various surfactant-based solid dosage forms, and nanoparticle-based suspension/solid) are employed to solve a major challenge in oral bio-availability, the *in vitro* screening methods that are applicable vary with the formulation type and so results are often difficult to compare across different formulation designs.

As mentioned above, dissolution data may be difficult to interpret in the absence of *in vivo* data. One such example is shown in Figure 7, for a Merck development compound. Compound E is a weak base, dosed as the hydrochloride salt, with high solubility at $\text{pH} < 2$. Its solubility drops drastically to less than $1 \mu\text{g/mL}$ at the intestinal pH range. In an effort to assess whether food effects on absorption occur clinically, dissolution of compound E was conducted

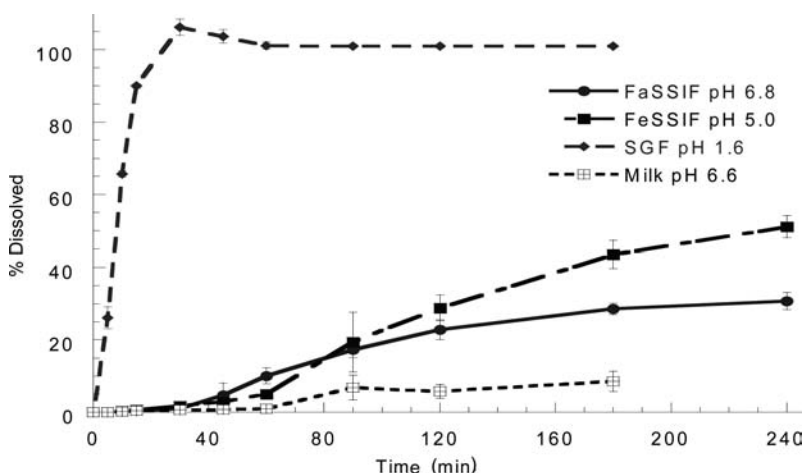


FIGURE 7 Dissolution of the bis-hydrochloride salt of weak base development compound E in biorelevant media to simulate fasted- and fed-state administration (500 mL, USP II, 100 rpm).

in four media to simulate the fasted and fed stomach and intestinal contents. Dissolution data appeared to correlate well with the pH-solubility profile of the compound, with fast and complete dissolution in the SGF media and better dissolution in FeSSIF (pH 5) compared with FaSSIF (pH 6.8). The results offer a somewhat contradictory picture in terms of solubilization in the stomach versus intestine with and without food. Solubilization in the stomach would be favored in the fasted state, while the opposite would be the case for the intestine. Under intestinal conditions, the difference in percentage release from the *in vitro* tests only becomes apparent after the two-hour time point. Subsequent clinical data exhibited a significantly positive food effect for compound E, indicating that the intestinal effects predominated for this compound.

It is also not uncommon to observe discrepancies between *in vitro* data and results in preclinical animal models. Compound F is a BCS class II weak base with moderate solubility (~ 200 $\mu\text{g/mL}$) at pH 2 but less than 1 $\mu\text{g/mL}$ in the intestinal pH range. Dissolution was carried out using USP apparatus IV, with 0.1 N HCl as the dissolution medium. The dissolution method had been previously shown to provide a good correlation to dog pharmacokinetic data for a series of LFCs. However when solid formulations were screened, a discrepancy was observed between the *in vitro* data and the dog *in vivo* study outcome. Specifically, three formulations, API-excipient dry blend-filled capsules (DFCs) 1, 2, and 3, were tested *in vitro* and showed percentage dissolutions of 71%, 20%, and 83%, respectively. Although DFC 3 provided somewhat higher exposure in the dog pharmacokinetic study than the other capsule formulations (AUC 1.29 $\mu\text{M}\cdot\text{hr}$), DFC 2 also provided reasonable bioperformance (AUC 0.96 $\mu\text{M}\cdot\text{hr}$), whereas significantly lower exposures were achieved with DFC 1 (AUC 0.41 $\mu\text{M}\cdot\text{hr}$). While no clinical data are available to confirm the results of the screen, this example clearly illustrates the challenges faced by researchers on interpreting *in vitro* dissolution data, juxtaposing them on data in preclinical animal models and coming up with recommendations regarding clinical formulation selection.

In summary, the field of biorelevant *in vitro* dissolution as a formulation development tool is still evolving. Nevertheless, despite their potential shortcomings, *in vitro* tools such as dissolution are increasingly becoming the first line of screening during the formulation selection process. As more insight is gained through the increased implementation of such tools, it is also expected that predictability will continuously improve.

EVALUATIONS OF IMMEDIATE-RELEASE ORAL FORMULATIONS IN ANIMALS

In vivo evaluations of potential clinical formulations are often time-consuming and labor-intensive. Whenever possible, *in vitro* testing should be used as the initial screening tool to eliminate poorly behaved formulations. However, confidence in using *in vitro* data alone to make formulation decision relies heavily on the proper design of the *in vitro* tests and good understanding of the rate-limiting step to oral absorption. While *in vitro* characterization described in section "In Vitro Screening of Immediate-Release Oral Formulations" can serve as a powerful tool for clinical formulation screening, quality *in vitro*–*in vivo* relationship are not generated for many formulations containing

poorly water-soluble compounds. In vivo evaluations should serve as a complementary tool to the in vitro methods to achieve the optimal balance between development speed and performance.

Importance of Characterization in Animals

One of the primary goals of the in vivo evaluations is to ensure good bioperformance of the proposed clinical formulation. It should be noted that the primary purpose of in vivo evaluation of a proposed clinical formulation is not necessarily to accurately determine absolute oral bioavailability. Although such information would of course be useful for assessing the opportunity for further improvement of in vivo performance, the significant differences in ADME (absorption-distribution-metabolism-excretion) properties seen across species hampers prediction of human pharmacokinetics. Thus, in vivo evaluations in the formulation development space has focused on assessing relative oral bioavailability of the potential clinical formulations. Establishment of a rank order of bioperformance using the total exposure as the primary endpoint is the goal in most cases. The underlying assumption is that the formulation, which provides the highest exposure in animal model(s), will also provide adequate exposure in humans. In general, this assumption would appear safe, since the primary oral absorption pathway for most pharmaceutical compounds is by the transcellular passive diffusion process, which is qualitatively similar across commonly used preclinical species. As a matter of fact, good correlation of intestinal permeability has been demonstrated between rat and human (43,44) as well as between monkey and human (45).

In an industrial setting, in vivo evaluations of oral IR formulations usually take place in parallel or in tandem to in vitro screening. Depending on the formulation development stages, in vivo evaluation in preclinical models can at least serve the following purposes: (i) establishing a rank order among several potential formulations derived from diverse formulation processes and technologies; hence guiding formulation development efforts; (ii) qualifying the bioperformance of a proposed lead clinical formulation to ensure adequate exposure is achieved in humans; (iii) establishing comparable exposure between early and late-stage clinical formulations to facilitate formulation bridging; and (iv) quantifying magnitude of food effect and its dependency on formulation.

As discussed in section "In Vitro Screening Of Immediate-Release Oral Formulations," if the screening involves only conventional formulations (e.g., WG or RC tablets with or without surfactants), in vitro tests should be employed prior to the conduct of in vivo evaluations. For many BCS class II compounds, attempts should be made to establish an IVIVR so that the in vitro dissolution test can be used with increased confidence to identify the lead formulation. However, various in vitro methods would be needed for screening formulations derived from very different designs (e.g., lipid-based emulsion, polymer-based solid dispersion, and conventional WG or RC tablets with surfactants). The data from these diverse tests are often difficult to compare to effectively eliminate poor formulations but not to prematurely abandon a promising formulation. In these cases, in vivo evaluations can serve as a second level test to differentiate the various formulations. When disagreement in rank order is observed between in vitro and in vivo data, efforts should be made to understand the discrepancy.

Common Animal Models for Assessing Oral Clinical Formulations

In theory, all common preclinical species can be used to study the bio-performance of solutions and suspensions as clinical formulation candidates, similar to the evaluation of formulations used in toxicological studies. Despite a good correlation between rat and human intestinal permeability, the rat (one of the most commonly used species for toxicology formulation assessment) is not typically used for clinical formulation evaluations since the small GI dimensions restrict use of solid dosage forms. The use of specialized approaches such as the Torpac[®] minicapsule kit has enabled the dosing of dry-filled capsules or LFCs to rodents (46,47). However, limited data are available in the open literature on the utility of this approach. Furthermore, because of continuous bile recirculation in the rat, it is unclear whether the dissolution process can be considered equivalent to that seen in larger animals and in humans.

The vast majority of published data on evaluations of oral clinical IR formulations are generated in either dogs or NHPs. The dog (especially Beagles) has often been the species of choice for *in vivo* evaluation of oral clinical IR formulations. Several characteristics of dogs have led to its preferred status: (i) similar GI dimensions to humans that allows easy dosing of common dosage forms (48,49); (ii) sufficient body weight (10–15 kg) for dose scaling on a mg/kg basis; (iii) ease of handling and dosing under either fasted or fed conditions; and (iv) large number of literature examples for a variety of formulations (50–54). However, the dog as an oral absorption model for clinical formulation evaluations has its limitations, which should be taken into consideration during the design of the study as well as the interpretation of pharmacokinetic data. Some of the known differences between dog and human relevant to studying oral drug absorption include: (i) variable and low gastric acid output in fasted state, leading to a wide range of and often higher gastric pH than humans (55–57); (ii) about one-half of small intestinal transit time of humans in the fasted state (58,59), derived from the difference in small intestinal length between species (6.25 m in humans vs. 3.24 ± 0.09 m in Beagle dogs, $N = 8$) [Merck unpublished data, (49)] leading to potential incomplete absorption or inaccurate assessment of human absorption of compounds that only absorbed in the upper GI tract; (iii) higher bile output in response to meals and different bile composition (48,60) leading to potential overestimation of solubility in human GI tract; and (iv) lack of a strong correlation between dog and human intestinal permeability (61,62).

Both rhesus and cynomolgus monkeys have been reported as suitable models for studying human clinical formulations (45,48,63). Several GI features provide a basis for the use of monkey model: (i) GI dimensions somewhat smaller than human but not precluding dosing of common dosage forms (48); (ii) relatively similar gastric pH and small intestinal transit time to humans (63); and (iii) strong correlation with humans for intestinal permeability (45). NHPs are also often used as the nonrodent species for toxicology studies in preclinical development, leading to a rich *in vivo* data set with a variety of liquid formulations. Some clear limitations of the monkey model have been listed as (i) dosing-induced stress, leading to shut-down of gastric acid secretion and/or variable pharmacokinetic data; (ii) difficulties in conducting food effect studies in terms of ease of dosing of test meals and meal types that can be used; (iii) slow return to baseline gastric pH (64); and (iv) relatively lower body weight for dose

scaling. Despite these challenges and limitations, the monkey has been the second most commonly used animal model for screening clinical formulations.

Recently, minipigs (e.g., Yucatan, Göttingen) have attracted increased attention as an alternative model for evaluation of clinical formulations (65,66). It has been reported that the minipig resembles the human situation better than any other nonprimate mammalian species with respect to eating behavior, anatomy and physiology of the GI tract (49). The reported small intestinal transit time of 3 to 4 hours and total transit time in the order of 24 to 48 hours for the pellets are very similar to humans (67). In addition, pigs have relatively similar body weight to humans (e.g., 50 kg for adult Yucatan minipigs vs. 70 kg for average adult males), hence no dose scaling is needed. However, pigs also have some clear limitations as a model for studying clinical formulations. For example, gastric emptying in pigs is somewhat slower than human, and is variable (67,68). For minipigs, it has been reported that the gastric emptying is size-dependent for nondisintegrating units (69). This feature is not only a major concern for the evaluation of erodible matrix CR formulation, but in our own experience also has shown high variability in IR formulation performance (Merck unpublished data). In fact, on the basis of their studies of gastric emptying of tablets and granules in humans, dog, and minipigs, Aoyagi et al. claimed that dog is a better animal model for oral bioavailability studies under fasted conditions than the pig (68). Nevertheless, as the minipig becomes more common as a safety assessment species for toxicological evaluations of pharmaceutical candidate compounds (70), it is anticipated that the use of this model for the evaluation of clinical formulations will increase in the near future.

Animal Screening of Oral Clinical Formulations

As discussed in section "In Vitro Screening Of Immediate-Release Oral Formulations," in vitro screening of prototype formulations should always take place prior to labor-intensive in vivo screening to avoid the evaluation of sub-optimal formulations and to conserve resources. Furthermore, applications of BCS-based biowaiver principles should be considered during formulation screening and internal decision-making. Evidence of rapid dissolution (e.g., >85% dissolved within 30 minutes) in SGF for IR formulations containing BCS class I or III compounds should be sufficient for qualification of good bio-performance without requiring additional in vivo testing and can therefore serve as supporting data for formulation selection. For compounds which exhibit very high aqueous solubility at normal fasted human gastric pH (i.e., pH < 3) but lower solubility at intestinal pH (e.g., a salt formed by a weak base and a strong acid), rapid in vitro dissolution demonstrated in SGF for conventional solid dosage forms can also provide strong indication that the formulation of interest is equivalent to an oral solution formulation, hence a biowaiver can be warranted for internal qualification. Expected variability in fasted-state stomach pH of the intended clinical population and the actual pH-solubility profile of the compound around the pK_a region should also be taken into consideration for the weak bases. For FIM studies that are typically conducted in healthy volunteers this would appear to be less of a concern.

This practice can be illustrated with the following example on the basis of our own experience. For the development of the FIM formulation of a weakly basic compound G, the API form used was the amorphous mono-hydrochloric

acid salt which is highly soluble in water (e.g., solubility of >300 mg/mL at a native pH of 1.07). The high solubility at pH < 3 would allow for complete solubilization of the highest oral dose for phase I studies. In vitro test results indicated that a simple roller compaction-based tablet exhibits fast dissolution in SGF media (~90% released in 10 minutes). Although the Caco-2 permeability value (15.5×10^{-6} cm/sec) for compound G is lower than the high permeability marker metoprolol (30.6×10^{-6} cm/sec), it is substantially higher than that of other low/moderate permeability compounds. Despite the formal BCS IV classification based on in vitro permeability data and low aqueous solubility at intestinal pH, fast dissolving formulations of compound G were expected to behave similar to an oral solution when dosed in the fasted state because of its high solubility at gastric pH. In fact, a solution formulation of compound G resulted in high oral bioavailability in rats at doses of 10 and 50 mg/kg (73% and 88%) in early toxicology studies, indicating high in vivo permeability. It was concluded that compound G exhibits BCS class I-like behavior in vivo if a fast dissolving oral formulation is dosed. Thus animal studies were not pursued and formulation was qualified on the basis of solely in vitro data. Furthermore, absorption simulations conducted in GastroPlus indicated that the impact of potential in vivo precipitation in small intestine on absorption would be insignificant and suggested a low probability of observing a food effect. In the subsequent phase I single ascending dose study, the roller compaction-based tablets demonstrated rapid absorption and excellent dose proportionality. The clinical data also confirmed the lack of a food effect.

In many cases, however, conducting preclinical pharmacokinetic evaluations of simple FIM formulations or prototype late-phase formulations is necessary to assure adequate exposures in humans. For practical reasons as well as the desire for fast turnaround of pharmacokinetic data, it is highly desirable to conduct in vivo studies involving five to six animals in a randomized full-crossover design. In some rare cases where high variability of the compound is known, more than six animals may be needed to differentiate formulations. To minimize the interanimal variability associated with in vivo studies, the study animals should have similar age and body weight. Doses used in animal studies are often scaled down on the basis of the relative body weight of humans and animals. Although a full-crossover design is highly desirable to minimize intra- and interanimal variability, noncrossover studies can be conducted in as few as three animals per group if low pharmacokinetic variability is observed and the timeline is short. For noncrossover studies, it is ideal to have customized formulations whenever possible so that the same mg/kg dose can be applied to each animal. This can easily be achieved for solution, suspension, dry-filled capsule and LFC formulations. For tablet formulations, this approach may not be practical, depending on the intended dose and drug loading used in the formulation. In such cases, dose-normalized exposure should be used when comparing the bioperformance of various formulations. Proper dose selection for in vivo screening becomes even more important if the compound exhibits nonlinear pharmacokinetics or if the objective of the study is to assess food effects on absorption. In such cases, maintaining the same dose in subsequent studies would be essential for meaningful data comparison.

To further minimize interanimal variability in in vivo screening of formulations containing poorly water-soluble compounds, the volume of dosing water or vehicle needs to be standardized and controlled to ensure that the same

volume of fluid is administered to each animal, similar to the situation in a clinical study. On the basis of the standard dosing volume of 240 mL of water for a 70 kg human, a body weight normalized water volume of about 3.5 mL/kg can be considered appropriate in animal dosing. In addition, to control the gastric fluid volume in each animal, access to water should be restricted within the first hour post dosing. When nonaqueous vehicles are used for a solution or suspension formulations, the maximal dosing volume should be based on the safety and tolerability of each vehicle.

As alluded to in section "Common Strategies for Developing Clinical Formulations," the increasingly popular use of simple formulations for FIM studies has some implications for achieving desirable bioperformance, especially for poorly water-soluble compounds. To speed up the process, optimal API form and formulation excipient selections are typically not possible. In vitro and in vivo screening is often conducted in parallel or no in vivo evaluation is performed at all to "save" time and resources. The major concern is whether rapid and abbreviated formulation development will lead to compromised bioperformance. It should be noted that a phase I single rising dose study typically covers a wide range of doses (e.g., 5–1000 mg). To achieve targeted pharmacokinetic and pharmacodynamic outcome, dose proportional increase in exposure is highly desirable. Poor bioperformance in the clinic can represent a significant hurdle for the program and put increasing pressure on formulation scientists to develop a better formulation under compressed time lines to allow for the program to go forward. One potential way to mitigate this risk is to qualify the selected FIM formulation in an animal model. A reference formulation, which is known to exhibit high oral bioavailability but may or may not be viable as a clinical formulation, can be tested against the proposed FIM formulation. A target relative oral bioavailability can be set as a qualification criterion.

This approach can be demonstrated in the example with Merck development compound B. As discussed in section "In Vitro Screening of Clinical Formulations," in vitro screening of mixtures of API with or without various surfactants including SLS, poloxamer 188, poloxamer 338, and Tween 80 was conducted for the BCS class II compound B, to assess the effect of surfactant on the size of API particles upon dispersion. The simple in vitro tests clearly indicated that the mixtures containing nonionic surfactants formed a well-dispersed and stable suspension with fine particles of large surface areas, which would be essential for the rapid in vivo dissolution and absorption of this BCS class II compound. When tablets containing the same nonionic surfactants were evaluated in dogs against the tablets without any surfactant, the rank order obtained in the in vitro tests was confirmed, that is, the tablet with higher molecular weight poloxamer and Tween 80 yielded the highest exposure while the tablet without any surfactant performed very poorly (Table 1). The exposure

TABLE 1 Exposure Data for Prototype Formulations of Compound B in Beagle Dogs at an Oral Dose of 10 mg/kg

Formulation	AUC _{0–24 hr} (μM × hr) (mean ± SD, N = 3–6)
Tablet with no surfactant	5.5 ± 2.5
Tablet with 1.5% SLS	4.6 ± 1.9
Tablet with 1% Tween 80	18.2 ± 8.7
Tablet with 1% poloxamer 407	35.9 ± 24.3

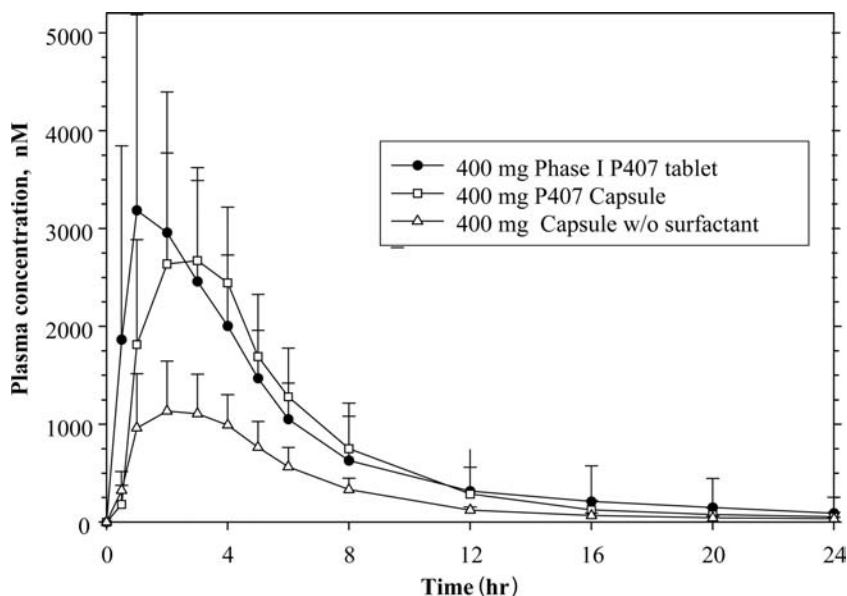


FIGURE 8 Mean pharmacokinetic profiles of compound B in healthy human subjects ($N = 6$) after oral administration of 400 mg of tablet or capsule formulation.

achieved with poloxamer 407-based tablet was comparable to that generated from the suspension formulation used in toxicological studies, which demonstrated dose proportional exposure up to doses much higher than 10 mg/kg. The poloxamer 407-based tablet was subsequently chosen as the FIM formulation based on the *in vitro* and animal data and the projected high clinical dose (requiring high drug loading). Follow-up clinical evaluations of the poloxamer-based 400-mg tablets (50% drug loading) or capsules (25% drug loading) showed a nearly two-fold increase in exposure compared with 400-mg capsules containing no surfactant (Fig. 8).

As previously discussed in section “Common Strategies for Developing Clinical Formulations,” employing simple formulations for FIM studies has also brought the challenge of formulation bridging after the development compound passes the initial clinical evaluations. While the dose range in phase I single- and multiple-dose studies can be very wide, it is often the case that the targeted doses for phase II and beyond fall within a relatively narrow range. Depending on the safe and efficacious doses identified for a given compound, the preferred formulation for mid-phase and late-phase clinical studies can be very different from the FIM simple formulation. Ideally, the relative oral bioavailability of a FIM formulation should be similar to the proposed late-phase formulation, so that a simple switch of clinical formulations can take place without dose adjustment. This is the preferred scenario from the clinical perspective, since any surprise in exposure change during a formulation switch could not only lead to potential delays in the clinical program but also pose a safety concern. In reality, the FIM formulation could differ significantly from the late-phase formulations in composition, configuration, process, and *in vitro* and *in vivo* characteristics. When this happens, dose adjustment may be required to maintain the exposure

established in a POC study. In the following example a simple LFC formulation of compound H was developed in a short time-frame to provide adequate exposure over a wide range of doses in phase I and phase IIa studies. The LFC formulation containing a solution of compound H in a lipid-based vehicle exhibited dose proportional increase in exposure and was well tolerated in all studies. As the compound progressed in clinical development, the efficacious human dose was found to be lower than projected (<10 mg). With the significant change of targeted clinical doses, formulation options were reevaluated for late-phase clinical trials and for commercial product development. For this BCS class II compound at the new, lower target dose, a tablet formulation containing micronized API and a common surfactant prepared by conventional roller compaction process became the preferred option for product development. The simpler formulation demonstrated good bioperformance in animals and humans. However, the bioinequivalence between the LFC and the tablet formulations required a dose adjustment for subsequent clinical studies.

Specific Considerations for the Dog Model

The differences in fasted gastric pH between dogs and humans should have little or no impact on the screening of formulations containing neutral or highly permeable weak acidic compounds. In contrast, the higher gastric pH in the fasted dogs is particularly important when evaluating formulations of weak basic compounds. Regardless of the API form used in the formulation (i.e., a free base vs. a salt formed with an acid), in vivo precipitation of the API is anticipated during the transit of the dissolved formulation to the upper small intestine. The gastric acid output or pH can have a significant impact on the stability or extent of gastric solubilization of API and the kinetics of in vivo precipitation upon gastric emptying. In our experience, fasted dogs often yield lower and variable exposures for the same weak base formulation than observed in humans. A potential solution to this limitation is the pretreatment of fasted dogs with an intramuscular injection of pentagastrin, an analogue of gastrin that reproducibly stimulates gastric acid secretion in human and animals (50,57).

Use of pentagastrin treated dogs for formulation development is a relatively common practice. An example is given by Badawy et al. (40). Specifically, the authors utilized pentagastrin or famotidine pretreatment of dogs to understand the gastric pH effect on oral bioavailability for a poorly water-soluble weak base compound, BMS-561389, and to develop a solid formulation strategy to overcome this gastric pH interaction. The lead formulation (a tablet containing extragranular tartaric acid), selected from in vitro dissolution studies (discussed in section "In Vitro Screening of Clinical Formulations"), was further evaluated in a dog model for pH-dependent absorption. Drug absorption from the tablet containing tartaric acid was substantially independent of gastric pH in the canine model. The control tablets, which are well absorbed in fasted humans, also performed well in dogs pretreated with pentagastrin. However, pretreatment with famotidine (40 mg) decreased the $C_{\max}$ of the control tablet in dogs by 85% and reduced its $AUC_{0-24 \text{ hr}}$ by 88% (Table 2). These data also confirmed the rational selection of the lead formulation based on the in vitro dissolution tests. In this case, a multitier approach was successful in identifying a solid dosage form that minimizes the pH-dependent absorption of this drug candidate.

In another report by Knupp et al. (54), the pentagastrin-pretreated dog model was effectively employed in the development of clinical formulations of

TABLE 2 Pharmacokinetic Parameters of BMS-561389 in Dogs After Administration of 100-mg Tablets

	AUC (ng·hr/mL)	C _{max} (ng/mL)
Control tablet (pentagastrin administration)	10,716	2413
Control tablet (famotidine administration)	1,143	257
Tablet with 16.7% extragranular tartaric acid (pentagastrin administration)	14,408	2136
Tablet with 16.7% extragranular tartaric acid (famotidine administration)	10,270	1720

Source: From Ref. 40.

didanosine, a purine nucleoside analogue approved for the treatment of human immunodeficiency virus infection. Didanosine is an acid-labile drug, which is extremely unstable at pH values less than 3 and requires protection against gastric acid-induced hydrolysis. Beagle dogs pretreated with pentagastrin have been used to screen different didanosine formulations. The absolute bioavailability of didanosine from a saline solution decreased from approximately 43% in untreated dogs to 8% after pretreatment with pentagastrin, confirming the instability of didanosine at lower gastric pH. Administration of a buffered solution of didanosine to untreated and pretreated dogs yielded bioavailability estimates of 37% and 30%, respectively. In humans, the bioavailability from a similar buffered solution was approximately 40%. Pentagastrin-pretreated dogs were then used to evaluate four new formulations relative to a citrate-phosphate buffer sachet, the formulation selected for large-scale clinical trials in humans. Two of these new formulations, a chewable tablet and an antacid suspension, were more bioavailable than the reference sachet in both dog and man, necessitating an adjustment in the dose of didanosine when administered as the chewable tablet. Overall, dogs pretreated with pentagastrin accurately predicted the improved bioavailability of new didanosine formulations prior to clinical use.

The ability to predict food effects on absorption in humans from animal models could accelerate product development by avoiding formulation redevelopment in cases where such an effect is observed in phase I studies. Hence, proactive assessment of potential clinical food effect should be taken place as early as possible. However, the level of success of predicting human food effect based on preclinical food effect studies varies in the literature. Lentz et al. reported a beagle dog model capable of predicting a compound's potential for a human food effect (71). The final model employed a 50-g aliquot of the FDA proposed high-fat meal with the combination of pentagastrin pretreatment in the fasted state. The authors obtained good correlation between the magnitude of food effect seen in dogs and that seen in the clinic for the test compound set. It should be noted that the dog food effect model can be effectively used not only in lead optimization in late drug discovery to assess the liability of such an effect when compounds progress from discovery to preclinical development but also in screening of formulations during all phases of clinical development.

In our own experience with a large number of development compounds over several years, the dog has been in general an excellent model for mimicking human food effect. One successful example was in the development of clinical formulations of MK-0869 (72). MK-0869 (aprepitant), a potent substance P antagonist, is the active ingredient of EMEND[®] which was approved for the

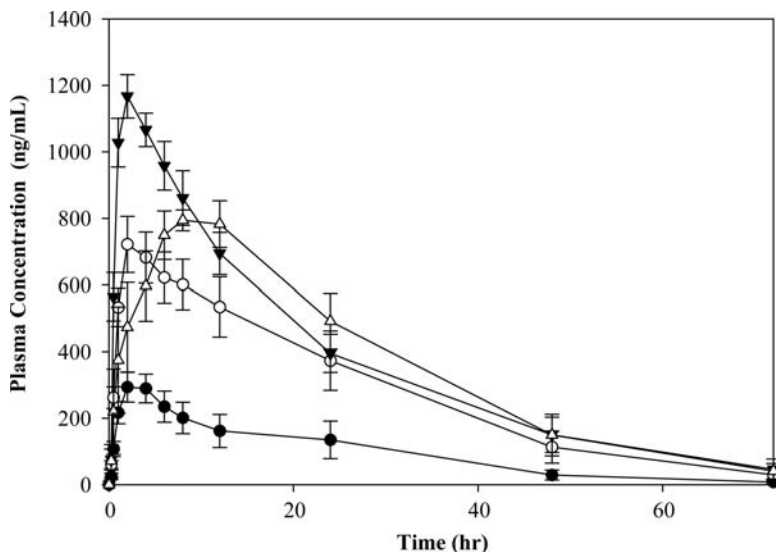


FIGURE 9 Comparison of mean ($\pm$ SE) plasma concentrations of MK-0869 following oral administrations in Beagle dogs ($N = 5$) of a conventional suspension (—●—, fasted; —○—, fed) and a NanoCrystal[®] dispersion formulation (—▼—, fasted; —△—, fed) of MK-0869 at a dose of 2 mg/kg. *Source:* From Ref. 72.

prevention of chemotherapy-induced nausea and vomiting. Early clinical tablet formulations of MK-0869 showed significant food effects on absorption, suggesting that formulation could have a significant role in improving bioavailability. A Beagle dog model was developed in an effort to guide novel formulation development. Using the suspension of the micronized bulk drug used for the tablet formulations, a food effect on absorption was confirmed in the dog at a similar magnitude to that observed in humans. Further dog studies demonstrated a clear correlation between particle size and in vivo exposures, with the nanoparticle (NanoCrystal[®]) colloidal dispersion formulation providing the highest exposure. The NanoCrystal dispersion also eliminated the food effect on oral absorption in the dog at a dose of 2 mg/kg (Fig. 9). In addition, the dog model was used for optimizing formulation processes in which the nanoparticles were incorporated into solid dosage forms, and for selecting excipients to effectively redisperse the nanoparticles from the dosage units. The human pharmacokinetic data using the nanoparticle formulation showed excellent correlations with those generated in the dog.

Challenges and Limitations of Animal Models

The physiological and biochemical differences between the GI tracts of human and commonly used lab animals present an inherent limitation to clinical formulation screening in animals. Additional complexity comes from the wide range of clinical formulations, which can contain high level of lipids, polymers, solvents, and surfactants. These combinations have created tremendous challenges in reliably comparing formulations made from different processes/technologies and forecasting bioperformance of the selected lead formulation(s) in human.

The challenges in translating data from preclinical species to humans is illustrated by the case of Merck development compound I. Compound I was a BCS class IV compound for which the early clinical formulation was a self-emulsifying LFC containing a solution of the API in a mixture of Imwitor 742 and Tween 80. The LFC formulation exhibited a positive food effect in human at a dose of 25 mg and above. In an attempt to mitigate the food effect, a second LFC containing a solution of the same API in a quaternary mixture of lipids and surfactants was developed. Supported by data from *in vitro* dispersion tests in SGFs, mono-, di-, and triglycerides of medium-chain fatty acids as well as two different types of surfactants in the second LFC were included to form a microemulsion upon oral dosing, to minimize *in vivo* precipitation, and to enhance the overall oral bioavailability in the fasted state. When the two LFCs were evaluated in the fasted and fed dogs at a dose of 1 mg/kg in a full-crossover study, the early LFC showed a 8-fold positive food effect while the second LFC reduced the food effect by 50%. The reduced food effect by the second LFC in the dog was primarily due to the significant increase (three-fold) in the exposure generated in the fasted state since minimal differences in exposure were observed in the fed state. The two LFCs were further evaluated in humans under fasted and fed (high-fat meal 827 kcal with 57% from fat or light meal 675 kcal with 25% to 30% from fat) conditions. In contrast to the encouraging data acquired in the dog model, no significant reduction of the food effect was observed in humans with the second LFC. It is also interesting to note that the second LFC yielded only a marginally higher exposure (1.3-fold) in fasted rhesus monkeys despite a three-fold increase in exposure in fasted beagle dogs. It is unclear why the significant reduction of a positive food effect was not realized in the humans in this case. Potential reasons that led to this unexpected outcome of the clinical evaluation include (i) differences in rate of digestion of various lipids used in the two formulations in the two species; and (ii) differences in types and concentration of bile salts in fasted dogs and humans.

Similarly, there are cases where accurate assessment of food effect in human using animal models can be complicated by uncertainty in dose selection and changes in formulations during the drug development process. In the report by Paulson et al. (73), food effect on oral absorption of a neat API-filled gelatin capsule formulation of celecoxib, a BCS class II drug, was evaluated in a dog model during early formulation development. The absolute bioavailability of celecoxib was higher when given as a solution formulation (64–88%) compared with the drug-in-capsule formulation (22–40%). The absorption of celecoxib in dogs given in a capsule was delayed by food, although systemic exposure increased by two- to three-fold at a dose of 5 mg/kg after a low, medium, or high-fat diet. Unlike dogs, celecoxib given to humans at a dose of 200 mg as a formulated capsule formulation with a high-fat meal exhibits only a slight increase in $AUC_{0-\infty}$ (11%) that is not clinically significant with regard to safety or efficacy. The authors attributed the insignificant food effect in human to the lower dose and longer GI residence time, both of which may promote absorption of celecoxib. The food effect studies in the dogs and humans were also conducted according to different protocols (although the same diet was given to both dogs and humans in the high-fat meal studies), which could also have contributed to the differences in the magnitude of the absorption response to food. Specifically, the healthy subjects received a 200-mg dose (2.85 mg/kg for a 70-kg body weight) and the dogs received 5 mg/kg (35–70 mg depending on

body weight). In addition, the dogs were given the celecoxib as neat drug in capsule and the healthy subjects were administered a formulated capsule, although it should be noted that the formulations showed no difference in oral bioavailability in dogs.

It worth mentioning that even though the commonly used pentagastrin pretreatment in dog may be a solution for normalizing dog gastric pH to a lower value, our experience suggests (Merck unpublished data) that the resultant pH is likely to be lower than the values typically observed in healthy human volunteers (74). This artificial pH-fix approach can therefore over-predict dissolution of poorly soluble weak bases, leading to an over-estimate of bioperformance.

In summary, there is no perfect preclinical model that can truly mimic the experience of an oral IR formulation in human GI tract. Despite various shortcomings and limitations, in our experience, the dog has often been a reliable model for establishing rank order among various clinical formulations as well as a good predictor of clinical positive food effect.

FUTURE OPPORTUNITIES

As the landscape of pharmaceutical industry changes, formulation screening and development process will continue to evolve accordingly. It is anticipated that formulation development time shorter than the current average will be used to bring a lead compound from drug discovery to clinical evaluation. It is also anticipated that significant and continued efforts will be made by pharmaceutical companies to reduce the time required from the end of POC studies to worldwide product registration. However, there are many external and internal hurdles that pharmaceutical companies will have to face or to resolve to achieve both goals.

Streamlining Work Flow in Formulation Development

It is not unusual nowadays for a typical pharmaceutical company to have R&D facilities located on several continents. Whether a company chooses a centralized or an autonomous operating model, the complexity of drug development has forced companies to structure their activities in a compartmental hierarchy. The formulation development and screening activities shown in Scheme 2 can be supported by multiple departments at a single development site or across several sites around the globe. For early formulation development, formulation activities in Pharm R&D are intimately linked with API supplies and PK/PD evaluations. These are often provided by different departments or organizations within a company. For late-phase formulation development, additional organizational structures will be involved, including commercial manufacturing and marketing. Misalignment of priority and redundant development efforts can occur because of lack of clear work flow, overlapping roles and responsibilities, and/or ineffective communication channels. In addition, fear of failure or lack of risk-taking often leads to the conduct of excessive in vitro and/or in vivo testing of proposed formulations prior to clinical evaluations. The fear of failure becomes more prominent during formulation changes in the late-phase clinical evaluations because of the potential high business impact. A recent article by Cook et al. highlighted the current state in the industry, which has failed to take advantage of BCS-based biowaivers for BCS class I drugs since the FDA guidance came into effect in 2003 (75).

While it is not likely that the global operation of formulation development and evaluation is going to change significantly anytime soon, the key to effective operation is to have a culture of open communication and to streamline the process by minimizing organizational barriers. For example, effective transfer of information from preformulation physicochemical and biopharmaceutical characterization to formulation development is essential to the selection of formulation type (e.g., liquid vs. solid), process (e.g., wet vs. dry), and proper excipients (e.g., need for a wetting agent or surfactant to facilitate *in vivo* dissolution, an antioxidant to enhance chemical stability, or a polymer to stabilize amorphous form of API). Well-designed and organized *in vitro* biorelevant dissolution testing and *in vivo* evaluations can further enhance the ability to quickly identify lead clinical formulations. Cross-functional project teams do provide a mechanism for communication, collaboration, and alignment of common goals across a large company, but empowerment of the team can further maximize the chances of solving what can be very challenging technical issues.

Harmonization of Strategies and Methodologies

One of many challenges in a global pharmaceutical company is to identify best practices in every activity in formulation development process and to implement them across the multiple development groups and sites. It is not uncommon to observe that different development sites prefer certain formulation processes and technologies as well as *in vitro*/*in vivo* methodologies and tools. These personal or local preferences not only hinder the transfer of projects across sites, but also make data comparison and bridging rather difficult. Alignment of the formulation development strategy among the relevant functions, including prioritization of lead formulation technologies and processes as well as standardization of *in vitro* and *in vivo* testing methodologies, can have a profound impact on the efficiency of the NCE development process in a large Pharm R&D organization.

One example of the need for harmonization is to have the various groups and sites to agree on the method for determining the API permeability. In general, API permeability can be determined by various *in vitro* cell-based methods (e.g., Caco-2 cells or MDCK or LLC-PK-1 cells at a single or multiple pH values) or *in situ* methods (e.g., rat intestinal perfusion) during early formulation development. Each of these methods has its pros and cons with regard to predictability of human intestinal permeability, data variability between labs and operators, and resource requirements. The FDA BCS guidance's lack of mandate for any one permeability method or any prescribed set of experimental methods has been viewed favorably by the pharmaceutical industry to provide flexibility on permeability measurement. However, if an *in vitro* cell-based method is chosen, all development groups within a company should agree to select a common cell line and to develop a standard protocol for permeability determination. An alternative approach would be to centralize such testing in a single group or location so that permeability data for the entire development portfolio can be easily managed and data comparison will be most meaningful.

Other areas for potential standardization include *in vitro* dissolution testing and *in vivo* pharmacokinetic evaluations. In the last decade, many publications have demonstrated the applications of biorelevant dissolution tests to differentiate among potential clinical formulations (76–78). In many cases,

strong correlations were demonstrated between in vitro dissolution rate and extent of oral absorption. Nevertheless, traditional quality control-type dissolution testing for formulation screening under sink conditions is still common in many pharmaceutical companies, possibly because of the lack of confidence and experience in establishing IVIVCs. Similarly, harmonization of in vivo protocols would be necessary to facilitate data comparison between the lead and backup development compounds as well as during project or program transfers between groups or sites.

Novel and High-Throughput In Vitro Methodologies

As part of an effort to reduce the cycle time from lead optimization to FIM study, automation is being increasingly used in every key area of formulation development. High-throughput testing with good accuracy and reproducibility has been achieved in areas including API salt form (79) and polymorph screening (80). API physicochemical and biopharmaceutical characterization [e.g., pH-solubility profiles (81), in vitro permeability (82,83)], formulation preparation (e.g., Xcelodose[®] for neat API-filled capsules), and biological sample analysis [e.g., 96-well plate plasma sample extraction coupled with ultraperformance liquid chromatography with small particles (sub-2 μm) and monolithic chromatography LC/MS/MS (84)]. These advancements have allowed the completion of some tedious and routine tasks by robotic systems, and freed up precious manpower to conduct creative research.

To enable fast development of innovative formulations for poorly water-soluble compounds without increases in manpower, applying and expanding automation to areas that are traditionally operated primarily by manual methods will be essential. One of such areas is the conduct of routine in vitro tests for formulation screening and release including both sample preparation and dissolution testing. Dissolution tests for solid dosage forms are one of the most time-consuming and labor-intensive activities in a pharmaceutical analytical group. As technologies advance, a variety of options are available nowadays to either partially or fully automate each step of the dissolution process (85). The difference in turnaround time, amount of samples, and purpose of test between early and late-phase clinical formulation also dictates the level of automation appropriate for dissolution testing.

Biopharmaceutical Assessment in Interface of Drug Discovery and Drug Development

While the focus of traditional pharmaceutical evaluations in late-stage drug discovery is on physiochemical characterizations of APIs, a more detailed biopharmaceutical assessment of discovery lead compounds is gaining importance in pharmaceutical companies. The adaptation of BCS-based decision trees in early preclinical formulation development has echoed the use of the same principle in selecting lead compounds for development. The combined efforts of physiochemical and biopharmaceutical evaluations in late-stage drug discovery have enhanced the identification of drug candidates with better overall profiles as a developable pharmaceutical molecules.

Understanding of the sites of drug absorption in GI tract can not only signal the potential challenges in clinical formulation development but also allow the identification of discovery lead compounds that will have the maximal

potential for being developed as a once-a-day drug. This information can be highly valuable if the lead compound in development exhibits inadequate elimination half-life for once-a-day dosing. The intestinal-ported dog model can provide quantitative assessment of absorption at each segment of the intestine (86,87). If absorption is determined to take place primarily in upper small intestine (i.e., the compound possesses a narrow absorption window), a simple FIM formulation of a poorly soluble compound may exhibit poor dose proportionality in humans because of incomplete absorption and significant formulation development efforts might be required. On the other hand, if absorption is determined to occur efficiently over the entire GI tract, simple formulations could become viable options to conserve resources and reduce time to FIM dosing. Furthermore, good absorption in colon can also increase the probability of developing a once-a-day formulation such as a CR dosage form.

Similar to the contributions in clinical formulation development, the increased role of biopharmaceutics in the interface of drug discovery and early development can allow early assessment of potential food effect on absorption as a large number of discovery leads are poorly water-soluble compounds. Proactive and quantitative assessment of such a risk is essential for compound selection decision-making for certain therapeutic targets. Such assessment can be biorelevant in vitro tests, computer simulations, in vivo food effect studies, or the combination of the three.

Increase in the Use of Simulation and Modeling

During the past decade, the pharmaceutical industry has picked up significant momentum in introducing and implementing *in silico* methods as some of these models have become commercially available (e.g., GastroPlus: Simulations Plus; PK-Map, PK-Sim: Bayer Technology Services) (88–93). Many pharmaceutical companies have now integrated computer modeling as part of formulation development process. The timely and effective use of this approach can have significant impact on many aspects of formulation development including (i) the establishment of API delivery specification, (ii) proactive assessment of food effect, (iii) facilitation of biowaiver, and (iv) prediction of bioequivalence among formulations. Kuentz et al. (20) recently reported that as part of a strategy for cost-effective development of clinical formulation GastroPlus was used as a pharmacokinetic simulation tool in preclinical formulation development. In this example, GastroPlus was used to simulate the absorption process on the basis of preformulation data and to determine the sensitivity to changes of selected input values. The input data consisted of a physicochemical properties including solubility in simulated physiological media as well as permeability determination.

It should be emphasized that quality simulations are not possible without relevant and accurate input data, that is, the better the input, the better the output. For example, the most common input for GastroPlus software for oral absorption prediction includes API solubility across physiological pH range, API permeability, and in vitro dissolution profiles. If these data were to be generated under biorelevant conditions, they would provide more meaningful input and consequently better predictions. While in most cases in vitro permeability data such as Caco-2 permeability can provide an adequate initial assessment of human intestinal permeability, permeability data generated via

rat intestinal perfusion is preferred for a more accurate estimate. In vitro permeability with the Caco-2 assay underestimates in vivo permeability, as evidenced by the high oral bioavailability achieved in preclinical species with some compounds that have poor Caco-2 permeability values.

One potential opportunity for employing modeling in formulation development is to forecast bioequivalence or bioinequivalence between early and late-phase formulations. Effective use of this approach could aid risk assessment and increase confidence in decision-making, especially when conducting a formulation bridging study in human that has no regulatory impact. As mentioned previously, the critical input to such type of modeling activity is the in vitro dissolution profiles of the two formulations. However, this approach will not be easily adapted by a low-risk tolerance and experimental data-driving organization, even if the in vitro dissolution data has previously shown promising correlation with in vivo data.

There is no doubt that clinical formulation development will continue to be a complex process. The emergence of new business and operational models, such as outsourcing and partnerships, will further increase the complexity of the process. Recognizing any work flow and operating model for formulation development and screening will have its pros and cons, it is the duty of the pharmaceutical scientists, who should be open-minded about alternative approaches and methodologies, to collaborate and create innovative products.

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Computer Models for Predicting Drug Absorption

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INTRODUCTION

Within the last decade, computer-based absorption models have developed to the stage where they are now available as commercial software tools and are being used as a standard tool by scientists working in pharmaceutical research and development. Even before this, mathematical models had been helpful in developing our understanding of how physiological variables, physicochemical properties, and dose and formulation affect oral absorption. A simple early model combined the key compound properties of permeability, ionization, and solubility in an empirical equation delivering a number related to the absorption potential of a drug (1). In the next years numerous more complex models were developed, one of which provided the theoretical basis for the Biopharmaceutical Classification System (2), now widely used to aid decision taking in pharmaceutical formulation development and as the basis for regulatory decisions on biowaivers (3,4). An excellent review of many of the more useful early models has been provided by Yu et al. (5). These models related measurable drug properties and physiological parameters in mathematical formulae, and analytical solution of the equations provided insight into how absorption behavior was expected to change with drug properties. Such models did not rely on the computer. However, the models developed in the last decade are more complex, based on more realistic representations of gastrointestinal tract physiology, and able to track the simultaneous processes of drug transit, dissolution, and transport. As they are too complex for analytical solution they must be solved numerically on the computer to provide a dynamic simulation of the oral absorption process. The development and wider use of such models have clearly been encouraged by the exponential increases in desktop CPU power and today even the most complex simulations involving transit and dissolution of particles of varying sizes, with absorption, metabolism, and distribution of drug in the whole body can be run in seconds on a standard desktop PC.

Computer-based absorption models can assist research into mechanisms of drug absorption and so are useful in both academia and industry. Workers in academia often custom-build models using general purpose modeling software, for example, Stella (<http://www.iseesystems.com>) (6–9). However, for scientists in industry the convenience of a supported, user-friendly software package usually outweighs the cost and flexibility considerations that favor custom model building. To meet this need, several commercial absorption software tools are currently marketed. GastroPlusTM, based on Yu and Amidon's compartmental absorption and transit (CAT) model (5), was the first such commercial software to appear. Simulations Plus (<http://www.simulationsplus.com>) extended the CAT model and produced a more user-friendly software tool

adding features such as pH-dependent solubility and permeability. They renamed the new model the Advanced CAT or ACAT model (10). The Intellipharm software (<http://www.intellipharm.com>) builds on the mixing tank model of Dressman (11). It appeared not long after GastroPlus and has since been extended in several ways (12). An alternative to the CAT-based models is implemented in the PK-Sim[®] software (13). PK-Sim first emerged as a whole-body physiologically based disposition model, but later added an absorption component where the gastrointestinal tract is modeled as a continuous tube. More recently, two other software tools implementing absorption models, largely based on the ACAT model, have appeared from Cyprotex (<http://www.cyprotex.com>) and SimCYP (<http://www.simcyp.com>).

The intention of this chapter is to review how computer-based tools for simulation of oral absorption can be useful at different stages in pharmaceutical research and development. The emphasis is on reported applications of the currently available commercial software tools. Providing support to the examples, some background theory is first presented.

THEORETICAL BASIS OF THE MODELS

The ACAT Model

The theoretical basis and mathematical description of the CAT model is provided, in detail, in several papers from Yu (14–16), and further details of extensions made in the ACAT model are given in a paper from Simulations Plus (10) and in a book chapter (17). Briefly, the ACAT model includes nine compartments corresponding to different segments of the gastrointestinal tract. The compartments are linked in series, with the first compartment representing the stomach and subsequent compartments corresponding to duodenum, jejunum (2 compartments), ileum (3 compartments), cecum, and ascending colon. Compartment properties are set according to the known pH, volume, and permeability characteristics of the corresponding intestinal region. For an immediate-release formulation, the transit of drug material between the compartments is modeled as a first-order process, where the rate of drug mass transiting out of each compartment is proportional to the amount remaining in the compartment. In the original ACAT model compartment transit times were equivalent, but this constraint has been relaxed in more recent versions so that the transit times are now closer to physiological values for the corresponding regions. For each drug to be simulated, compound-specific input data for solubility, permeability, $\log P$, pK_a , particle size, and dose are fed into models of dissolution and absorption. For dissolution, a model based on the Nernst-Brunner modification of the Noyes-Whitney equation is implemented, where drug is treated as a set of particles and the dissolution rate in each compartment is modeled as proportional to the total surface area of all particles currently residing in that compartment and to the concentration difference between the particle surface and bulk solution. Absorption across the gastrointestinal mucosa is determined by the permeability of the drug. By definition, if P_{eff} is the effective permeability value measured using the regional human perfusion technique (18), then the absorption rate for a compartment would be proportional to P_{eff} , the mass of drug in the compartment, and the reciprocal of the radius of the intestinal region represented by the compartment. However, in practice, such P_{eff} values are available for just a few drugs and are usually only

measured in the jejunum. Usually the permeability of a research compound is measured in an *in vitro* experiment such as the parallel artificial membrane permeability assay (PAMPA) (19,20) or in Caco-2 cells (21). For use in the ACAT model, this data needs to be transformed to an estimate of human jejunal permeability. This transformation is based on a correlation built for those drugs where human permeability has been measured. The modeling of passive absorption from each compartment is further complicated by regional permeability changes due to both the changing mixture of transcellular and paracellular transport, the changing surface area due to different dimensions and density of villi and microvilli, and to the varying drug ionization with different regional pH. As no verified mechanistic model accounting for all these effects and allowing prediction of regional changes in permeability for a compound exists, GastroPlus provides a number of flexible options. A log D model scales the regional permeability so that as the ionized fraction of a compound increases the effective permeability decreases, and this model has been optimized to best match the human absorption data for a set of drugs with experimental human jejunal permeability values. The log D model provides a useful default when modeling a drug on the basis of a single estimated jejunal permeability value, but if more detailed measurements of regional variation in P_{eff} are available they may be entered for each compartment. The situation is further complicated when modeling absorption in animals, and this is dealt with by allowing input of species-specific permeability measured with the perfusion technique or, if this is not available, by providing a default scaling between the species permeability and human. Other capabilities of the ACAT model include modeling release from the dosage form for simulation of controlled release formulations, modeling of active efflux and uptake transport in the gut wall, modeling of intestinal metabolism, modeling of enterohepatic recirculation, and optimization of model parameters to match simulations to observed data.

In general, although the overall ACAT model structure is fixed, the model parameterization and specific model used for certain processes is flexible. For example, the pH, length, radius, and transit time of the compartments are all-accessible and can be changed, and solubility dependence on pH can be predicted using a Henderson–Hasselbalch model or can be specified as a multipoint profile of solubility versus pH. Such flexibility is important to support the use at various stages in the drug research and development process since the amount of available input data tends to increase as a drug progresses. Early simulations must make many assumptions and use default settings, while drug-specific models may be constructed for more advanced compounds.

Numerical integration of the linked differential equations describing all the processes included in the ACAT model yields the amount of drug absorbed into the portal vein as a function of time. For simulation of plasma profiles after oral dosing the predicted absorption versus time profile is used as input to a disposition model. In GastroPlus this is possible in either of two ways. If plasma concentrations measured after intravenous dosing are available then a compartmental model may be fit to these data and used to simulate disposition. This process is facilitated with a module that fits one-, two-, or three-compartment open models, recommends the best fit and performs automatic linking of the chosen model to the ACAT model. An alternative is available via a physiologically based pharmacokinetics (PBPK) module that provides a whole-body disposition model including options for prediction of tissue distribution and

metabolism scaling. For use of the PBPK model, essential inputs are lipophilicity, ionization constant, plasma protein binding, and estimates of hepatic (e.g., by scaling of in vitro data from microsomes or hepatocytes) and extra-hepatic clearance (e.g., renal filtration).

Other Models

Neither the Cyprotex nor the SimCYP model has yet been described in detail in the open literature but available information indicates that both are similar to the ACAT model. The Cyprotex model has just five compartments (22) while the SimCYP model has been named advanced dissolution absorption and metabolism (ADAM) (23) and differs from GastroPlus in that it implements a dissolution model devised by Wang and Flanagan (24). The SimCYP software was developed initially as a tool for in vitro to in vivo scaling of clearance and metabolic drug-drug interactions in human populations (25), but has now developed into a full whole-body physiologically based modeling tool including an absorption component. The "QGut" model included in SimCYP for intestinal first-pass effect has been described and validated (26). The name QGut derives from the flow term used in the model, which is a hybrid of both permeability through the enterocyte membrane and villous blood flow. When combined with an intrinsic metabolic clearance term derived from in vitro data the QGut model captures the fact that either a higher permeability through the enterocyte or a higher blood flow carrying drug away from the enterocyte will decrease first-pass exposure to metabolizing enzymes. The QGut model still assumes a single homogenous intestinal compartment and is now being extended to allow for transit and gradients of enzymes and transporters down the gastrointestinal tract.

The absorption model in PK-Sim, first described for the rat (27), has some similarity to the dispersion model published in the 1980s by Ni (28). The gut is modeled as a continuous tube and the drug concentration profile is described by a Gaussian function, which moves down the tube and spreads out with time mimicking the transport and dispersion of drug in the intestines. The function describing the position of the center and width of the distribution was fit to literature data on transit of a nonabsorbable marker. The initial paper on the rat model did not account for the pH dependence of solubility or permeability, but this was added in a subsequent paper describing adaptation of the model to human physiology (29). An interesting feature introduced in this paper was the prediction of human permeability from measured membrane affinity and compound molecular weight. When used in the human absorption model this calculated permeability delivered a good correlation to observed fraction absorbed for a set of 119 soluble training set compounds. The model continues to develop and most recently was adapted to the monkey (30).

The Intellipharm software is based on a single tank representation of the gastrointestinal tract with tracking of time-dependent changes to factors that affect the dissolution and absorption (12). Thus, to account for solubility changes because of the pH changes as drug transits through the gastrointestinal tract, the solubility term in the Noyes-Whitney equation is made time dependent. Similarly, the permeability is time dependent, and drug release can also be modeled. Strength of the Intellipharm software has been the modeling of dissolution rate resulting from different particle size distributions (31,32).

It is not possible here to provide comprehensive model descriptions or to list exhaustively the capabilities of the most recent versions of each tool. For this, the latest reference manuals are the best source of information and, while software developers may keep some aspects of the model proprietary, this cannot go too far since the software is being used to deliver mechanistic insights. Independent comparative reviews are limited and quickly become outdated. For example, a report from 2002 compared performance of GastroPlus to iDEA (33), but the iDEA software is now no longer available and GastroPlus has developed significantly in the years since this evaluation.

The rest of this chapter is devoted to examples of application of models.

APPLICATIONS WITHIN DRUG RESEARCH AND DISCOVERY

Modeling and simulation has been identified as a technology that will lead to productivity improvements in drug research and development (34). One benefit is the formal and realistic integration of data on physicochemical properties, pharmacokinetics, pharmacodynamics, formulation, and safety, which provides the basis for quantitative comparison of compounds and improved decision taking (35). Another advantage is that models act as a repository for the knowledge gained (36) and are key to translating preclinical knowledge to the clinic. As an example, a physiologically based model developed to simulate the absorption in preclinical species can be applied to predict human oral pharmacokinetics and then further refined during clinical development to allow a more mechanistic interpretation of clinical data, helping to explore hypotheses and guiding clinical formulation development. Figure 1 shows how the examples in this chapter cover different stages in research and development ranging from lead optimization during drug discovery through to phase 2 clinical studies.

Simulation in the Rat in Early Discovery

During drug discovery, considerable resources are required to assess the oral absorption of potential drug candidates in animals and there is interest in optimizing the use of such testing by applying simulation. Reliable simulations could help to reduce the number of animal experiments, a common objective of industry, regulatory, and advisory bodies (37), and could offer productivity

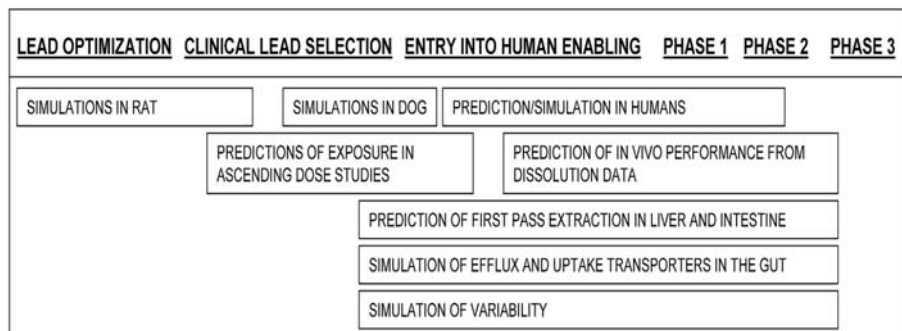


FIGURE 1 Applications of absorption modeling in drug discovery and development.

benefits during the optimization phase by decreasing the turnaround time for delivery of information to medicinal chemists. In a study performed in our group (38), simulations of oral profiles in the rat based on minimal *in vitro* and *in silico* inputs were compared to observed profiles for a set of 68 compounds taken from 6 chemical classes undergoing medicinal chemistry optimization in different drug discovery projects. The simulations were based on the screening data available at the early discovery stage. Aqueous thermodynamic solubility at pH 6.5 was measured in a high-throughput lyophilization assay (39) and permeability was measured in a PAMPA (19,20). The variation of solubility with different intestinal regions was assumed to depend only on the pH of that region and was calculated from the ionization predicted from *in silico* estimates of pK_a . Dissolution was calculated assuming a suspension of spherical particles all with initial radius of 1 μm and the first-pass effect was assumed to occur only in the liver and was calculated from the hepatic blood clearance predicted from scaling of *in vitro* data generated in hepatocytes. Typical for compounds at this early stage, the lipophilicity was high with a mean calculated $\log P$ of 3.9. Permeability was medium or high for all compounds while aqueous solubility was low with the average being only 37 $\mu\text{g/mL}$. The reliability of simulated absorption was assessed by comparing the predicted and observed bioavailability, and it was found that predicted bioavailability showed a strong bias to underprediction, which was associated with very low aqueous solubility. For 27 of 29 compounds with solubility less than 6 $\mu\text{g/mL}$, bioavailability was underpredicted. Assuming that the aqueous solubility was underestimating the solubility in the intestinal fluids, a correction was applied on the basis of a published theoretical relationship of bile salt solubilization to lipophilicity (40). This correction brought an improvement, although a large average prediction error of 31% remained.

In a similar evaluation, GastroPlus was used for the prediction of bioavailability in rat for 30 diverse compounds (41). The set of compounds used in this study showed high lipophilicity and low solubilities similar to the compounds used in our study, and the results were also similar with a root mean squared error of 32% when aqueous solubility was used as input for the simulations. The authors suggested that, to account for the influence of solubility enhancers in the dosed formulations (cyclodextrin or polyethylene glycol), the solubility in the dosing vehicle should be used as the relevant input. This approach did indeed deliver better results and reduced the error to 19%. However, such an approach probably needs further evaluation since the compounds mostly showed near-complete absorption and assuming complete absorption also gave a similar mean error of 22%.

These examples illustrate some of the limitations of absorption models applied during the early discovery stages, when only screening data with high intrinsic uncertainty is available to characterize the compounds. The first-pass effect estimated from intrinsic clearance in hepatocytes may be a source of significant error, especially when hepatic clearance is high, and can be underestimated if intestinal metabolism is important. Knowledge of particle sizes in dosed microsuspensions is typically not available and so dissolution limitations and formulation effects cannot be reliably estimated. In addition, the errors in *in silico* estimates of pK_a and $\log P$ can be large and lead to quite significant error in simulated exposures. Given these limitations, such early simulations often cannot be used to make reliable predictions of *in vivo* exposures. However, this

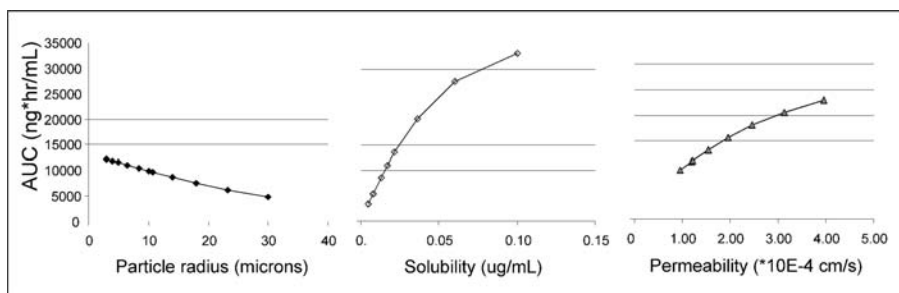


FIGURE 2 A parameter sensitivity analysis plot showing that small changes in solubility can have a large effect on simulated exposure in the rat. Exposure is measured as area under the concentration versus time curve.

does not mean that simulations are of no value. By integrating the available data, the model can be used to explore the sensitivity of absorption to different compound properties and can focus further experimental efforts onto the more critical factors (Fig. 2). Especially when the first in vivo data become available, hypotheses can be explored by changing input parameters to bring simulation and observation into better agreement. In addition further advances in in vitro measurements are already leading to earlier generation of more predictive data. For example, in our company, we now frequently obtain higher throughput solubility measurements in biorelevant media (42), which leads to more accurate simulations for low-aqueous solubility compounds.

Predict Exposures in Ascending Dose Studies

During the clinical lead selection phase only a few compounds are under consideration for progression to clinical development, and these compounds are more carefully characterized. Key questions are whether efficacious exposure in humans is achievable and whether adequate exposures in toxicological studies in animals can be achieved to ensure a good safety margin. As a part of an early assessment of the “developability” of a potential drug, a rough calculation of the maximum absorbable dose (MAD) is useful. The MAD concept was first outlined by Johnson (43), who developed an equation to estimate an upper limit of absorbed drug for neutral molecules with no dissolution limitation. Various modifications to the MAD calculation have been proposed (44) but simulations with physiologically based absorption models offer a more powerful way to explore absorption across a range of doses. A series of simulations run at a range of different doses can be used to examine how several different parameters (e.g., C_{max} , T_{max} , AUC, and bioavailability) are affected (Fig. 3). A comparison of the use of a simple equation for MAD to GastroPlus and a proprietary simulation program has been described by Ding and Rose (45). MAD predictions based on biorelevant solubility measurements and in silico permeability were compared to observed ascending dose data in human for a set of 20 compounds. It was found that the simulation approaches predicted higher MAD than the simple equations but still tended to underpredict the actual observed MAD. Our experience in applying GastroPlus for prediction of MAD in rat, monkey, and human has showed some examples where the simulation of solubility limitation

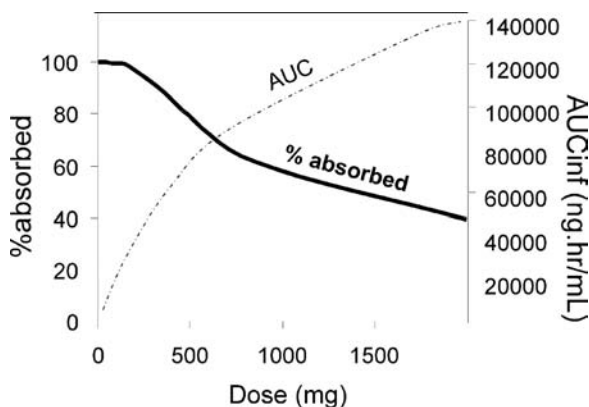


FIGURE 3 A parameter sensitivity analysis plot showing how the fraction of dose absorbed and exposure vary as the human dose is increased. Exposure is measured as area under the curve extrapolated to time infinity.

could be verified with *in vivo* data (46). However, clinical single ascending dose studies that extend to doses high enough to exhibit solubility limitation are quite rare, and while animal toxicological studies often extend to higher doses the validity of predictions based on solubility limitation is rather difficult to assess because of the likelihood of other nonlinearities in pharmacokinetics such as saturation of metabolism, nonlinear protein binding, or changes in elimination. Willmann applied an early version of the PK-Sim absorption model to match the dose dependence of chlorothiazide but concluded that extensions to the model to account for pH-dependent solubility were needed (27). A subsequent version of the PK-Sim human absorption model, including such an enhancement, was applied to a theoretical exploration of solubility limited absorption (29) but was not compared to observed data.

Assisting Preclinical Formulation Development

As a compound advances further in preclinical development and shows potential to proceed to human studies, the initial data generated in rat will be supplemented by *in vivo* data in a second species. At this stage formulation development work is undertaken both to achieve high exposures in toxicological studies as well as to determine appropriate approaches for clinical formulation. Dannenfelser and colleagues (47) described how GastroPlus can be used to help choose formulation approaches for a very poorly soluble compound with high permeability. Measurements in pure water showed solubility less than 1 $\mu\text{g/mL}$ but addition of bile salts produced a 10-fold increase and when a GastroPlus parameter sensitivity analysis was conducted it indicated that a significant increase in fraction absorbed could be expected if the *in vivo* solubility were increased. A subsequent experiment in dogs with a cosolvent-surfactant solution and a solid dispersion formulation confirmed a substantially better oral absorption of these formulations compared to a dry blend of micronized drug. The authors concluded that the simulations had provided an insight into the formulation development processes and allowed foresight to potential issues prior to formulation investigation.

A strategy incorporating GastroPlus as part of a biopharmaceutical characterization of a molecule has been described by Kuentz (48). In this study, GastroPlus was used to simulate absorption for a weekly basic BCS class II

molecule. Simulations were based on drug solubility measurements in bio-relevant media for the stomach and intestinal fluids and permeability was scaled from measurements in Caco-2 cells. A parameter sensitivity analysis in GastroPlus showed that changes of particle size and reference solubility over a technologically relevant range had minimal effect on the oral absorption. Fraction absorbed was predicted to remain almost complete since, although precipitation was expected on leaving the stomach, there was time for full redissolution and absorption during intestinal transit. In a second part of the study, a specially designed formulation screen in the dog looked at the performance of a worst case (capsule filled with the micronized drug) versus a best-case formulation (surfactant solution), and showed no clear difference in exposures. These findings, combined with the model simulations, resulted in the choice of the simpler formulation for "first in human" studies with associated cost savings.

Prediction of Oral Pharmacokinetics in Humans

A key task for preclinical research is to make a reliable prediction of human pharmacokinetics for compounds entering phase 1 studies. Absorption models that describe the gastrointestinal physiology for different species combined with *in vitro* data on biochemical differences have the potential to leverage accumulated preclinical knowledge and deliver an optimal prediction of absorption in human. Jones et al. developed a strategy for prediction of human oral pharmacokinetics using the ACAT model and tested it by comparing predictions to observed clinical data for 19 compounds (49). The strategy followed used comparison of simulations to observed data in preclinical species as a way to verify the validity of the assumptions made. Thus, provided the simulations in two or more preclinical species were accurate, a prediction to human was made using the same absorption parameters and under the same assumptions. In contrast to the limited data available as a basis for simulations made at an earlier phase in discovery, simulations at this phase benefit from a more complete and reliable set of input data. For the majority of compounds measured values for lipophilicity and ionization were available, and for compounds with low aqueous solubility measurements were often made in biorelevant media. The predictions were quantitatively assessed by comparing pharmacokinetic parameters derived from the simulated profiles to those based on observed data. This showed that predicted area under the curve was within twofold of the observed value for 76% of compounds. Time of maximal concentration was well predicted with 94% within twofold, but the maximal plasma concentrations were less well predicted with only 47% within twofold. The authors noted that availability of biorelevant solubility measurements seemed to be essential for reliable prediction of BCS class II compounds. The importance of permeability could not be reliably assessed since only one of the test compounds showed low permeability. Other interesting observations of this study were that the model-based predictions were more accurate than an empirical approach based on allometry and that the cycles of preclinical simulation and comparison to observed *in vivo* data performed during the process served as a significant learning opportunity. When simulations in animals were a poor match to observed data and the mismatch could not be easily explained through uncertainties in key input data, it was often the case that the prediction to human was

less accurate. This was attributed to lack of quantitative mechanistic models for some processes such as active transport and nonhepatic metabolism.

A similar study to evaluate GastroPlus as a tool for prediction of human absorption was described by De Buck and colleagues (50). On the basis of a similar approach, the predictive accuracy was assessed for 23 compounds dosed orally and was improved compared to that of Jones with 74% area under the curve predictions and 65% maximum concentration predictions within twofold of mean observed values. In addition, this study benefited from data obtained after intravenous dosing in human for 16 of the compounds, allowing a comparison of predicted and observed bioavailability. This showed 81% within twofold of observed values and a root mean squared error of only 15%. As with the Jones study, this study was limited in terms of poorly permeable compounds, although for two BCS class III compounds it was noted that data scaled from Caco-2 cells provided good prediction while *in silico* permeability did not.

Prediction of Food Effects

It is well known that the absorption of certain drugs is significantly affected by food. Such changes can lead to changed drug exposures for a given dose and thereby a change in safety or efficacy. For this reason the impact of food and dosing instructions relative to meals must be specified in the drug labeling (51). Also, as large variability because of food can impact the developability of a molecule, it is advantageous if compounds problematic in this regard are recognized early. Early model-based simulations of potential high food effect can assist in planning of clinical studies and bring mechanistic understanding to assist formulation work to reduce the food effect. A strategy for prediction of food effects based on GastroPlus models describing physiology in the fed and fasted states, together with biorelevant solubility and degradation data, has been described and illustrated for six compounds (52). The six compounds studied were all lipophilic, with log *P* values ranging from 2.3 to 6.5. Four of the compounds were weak bases and two were stronger bases. While all compounds showed moderate to high permeability, the aqueous solubility of four of the compounds was very low. FaSSiF and FeSSiF solubility was used as an input to reflect the changes in intestinal solubility with food, while the human ACAT model was adjusted to reflect food-related physiological changes in gastric pH, gastric emptying time, and liver blood flow. With food, the simulated gastric pH was changed from 1.7 to 5.0, average stomach residence time was changed from 0.25 to 1 hour, and hepatic blood flow was changed from 90 to 120 L/hr. In addition, the effect of the amount of fat in the diet was explored for two of the compounds, where the clinical study had included this factor. The only factor changed to reflect fat content was to measure solubility in a modified FeSSiF medium where the amount of oleic acid was increased. The results of the study showed that the simulations correctly predicted significant food effects (two compounds) and mild food effects (four compounds). For two poorly soluble weak bases, the positive food effect was explained as due to a combination of increased solubility due to both bile salts and the slowed gastric emptying, which provided more time for absorption. For a highly lipophilic weak base, a positive food effect was also correctly predicted and it was also possible to correctly simulate a dose-dependent increase in the ratio of exposures with high fat versus standard diet. This was explained as due to limited solubility, even in

the standard fed state, which resulted in dose-limited absorption at higher doses owing to an increase in the dose-to-solubility ratio. This study also showed the possibility of simulating the effect of pH-dependent drug degradation in the stomach based on *in vitro* data and noted that the dog showed similar food effects to humans for two of the tested compounds, but overpredicted the effect for a third compound. A separate study (53) used FaSSiF/FeSSiF data in GastroPlus to simulate the food effect in dogs and human for aprepitant, a lipophilic neutral compound with low solubility and moderate permeability. GastroPlus predicted qualitatively similar food effects in dog and human in line with observations (54). It seems that although the dog sometimes shows similar food effects to human there can be counter examples. One reason for differences is likely to be that solubility *in vivo* in dog and human are different. Measurements in dog intestinal fluid (55) have shown significant differences in the effect of food on intestinal solubility compared to human and it is clearly not always appropriate to use FaSSiF/FeSSiF media, which were designed for the human, as input for simulations in dog. So, although experience to date indicates that absorption modeling adds value to the use of animal models alone to predict food effects, an improved strategy would involve measurement of solubility in biorelevant media simulating the intestinal fluids in dog with incorporation of this data into the model (Fig. 4). Accurate simulation of food effect in the dog would then be used as the basis for a reliable simulation in the human, on the basis of FaSSiF and FeSSiF measurements.

Finally, progress in development of more appropriate biorelevant media should proceed hand-in-hand with absorption modeling because simulations based on solubilities measured in the new media can be compared to *in vivo* data to assess their relevance. This is illustrated in a recent paper where GastroPlus modeling studies were used to support the rationale for a modified recipe for fasted state simulating gastric fluid (56).

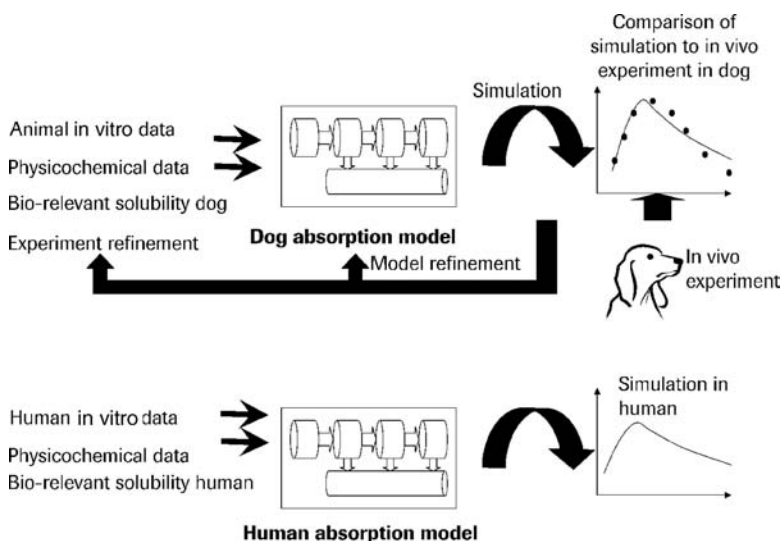


FIGURE 4 A proposed strategy for quantitative prediction of food effect in human using absorption models and biorelevant media simulating the gastrointestinal milieu in dog and human.

Predicting In Vivo Differences Due to Changed Dissolution

When developing a formulation it can be useful to be able to predict how absorption rate and hence plasma concentration versus time profiles will be affected by changes in particle size. Data on the particle size distribution of active pharmaceutical ingredient can be obtained, or dissolution rates can be measured in vitro. If phase 1 studies show that human pharmacokinetics appear to be suboptimal for a desired therapeutic effect, then it may be possible to change the oral profile via a modification of the drug release rate. Using the Intellipharma software, Johnson (12) validated a model for nifedipine absorption by correctly simulating the effect of particle size changes on plasma concentration profiles and then showed that the behavior of a controlled release formulation could be well predicted. Lukáčová (57) described how the default dissolution model in GastroPlus was used accurately to simulate differences in plasma concentrations in the dog for three formulations of cilostazol (58). The three formulations contained nanocrystals, jet-milled, and hammer-milled particles, respectively, and simply using their respective particle size distributions produced simulations matching the observed plasma profiles that differed by a factor of more than sixfold in exposures. Lukáčová also found that using the measured in vitro dissolution data as input to the simulation was not able to match the in vivo profiles, and this was attributed to a mismatch between sink conditions in vivo and in the in vitro dissolution test. GastroPlus was used to estimate the actual in vivo dissolution profile and a more realistic in vitro test was then proposed. Kesisoglou et al. (59) used GastroPlus to explore factors limiting absorption of a BCS class IV molecule intended to be administered at a high dose in the clinic. In spite of the poor permeability and potential for slow dissolution, a parameter sensitivity analysis showed that solubility was expected to be limiting, and this was partly confirmed when a study in dogs with two formulations differing widely in particle size showed no difference in exposures. It was concluded that modeling was useful to understand the rate limiting steps to absorption, predict the effect of drug properties on bioavailability, and help with setting specifications during the formulation development process. Lindahl et al. (60) used GastroPlus to simulate the plasma profiles of fluvastatin after single doses of immediate-release and extended-release formulations. The predicted good absorption from a 12-hour zero-order release formulation was in reasonable agreement with observed data and this was taken as an indicative that the prediction that 73% of the dose would be absorbed from the colon was realistic. As no efflux proteins were accounted for in the simulation, the authors suggested that efflux is most likely not an important factor for the overall absorption of fluvastatin. More recently, Wei et al. (61) used GastroPlus to establish an in vitro/in vivo relationship between particle size and clinically observed plasma time profiles for different glyburide products and concluded that in silico methods can assist the formulation scientist to set meaningful product specifications and could shorten the drug development process since appropriate biowaivers, on the basis of data from simulation studies, may be justified. Supporting this conclusion, a paper from Tubic-Grozdanis et al. (62) described how GastroPlus was used to simulate the absorption of several weak acid and weak base BCS class II compounds. They showed for a set of 10 drugs that simulation results for different formulations were in line with the results of bioequivalence studies and argued that prospective use of simulation could aid justification of biowaivers for selected BCS class II compounds.

Predicting First-Pass Extraction in Liver and Intestine

Accurate prediction of drug loss during the first-pass through the liver is important since, once drug has been absorbed across the gastrointestinal mucosa, this is the major factor determining overall bioavailability. In addition, it is recognized that metabolism in the gut wall can be a source of significant first-pass effect for drugs that are substrates of enzymes such as CYP3A4, which are expressed in the gut. Using GastroPlus, Agoram et al. (10) modeled the nonlinear hepatic first-pass effect for propranolol using saturable Michaelis–Menten clearance and explored the effect on bioavailability of changes in liver blood flow and protein binding. For midazolam, Agoram was able to scale Michaelis–Menten parameters measured in vitro in incubations of liver microsomes to the in vivo situation and simulated accurately the increase in bioavailability with dose. The model included extraction in both liver and intestine since midazolam is cleared by CYP3A4. Even though the expression level of CYP3A4 used in the model was 70 times less than in the liver, the extraction during the passage through the enterocytes was comparable to the hepatic extraction, which is in agreement with in vivo measurements. Using measured expression levels of CYP2D6, the same approach was applied for metoprolol and simulations accurately matched the known bioavailability. Yang et al. (26) demonstrated good predictions of fraction metabolized in the gut for 16 CYP3A substrates using the Qgut model in SimCYP. The predictions for 16 CYP3A substrates were best when it was assumed that no binding occurs in the enterocyte. In this case, the mean error in the fraction escaping gut metabolism was only 7%. Nonetheless, there were still some significant outliers in the predictions and the authors concluded that to provide more reliability, further complexities must be addressed by allowing for transit and gradients of enzymes, and transporters down the gastrointestinal tract.

Simulation of Efflux and Uptake Transporters in the Gut

Quantitative prediction of the effects of intestinal transporters on absorption of drugs still remains a challenge since knowledge of the expression patterns and protein abundances along the gastrointestinal tract is limited and the in vitro to in vivo scaling techniques have yet to be validated. However, it is clear that computer-based modeling will play an important role in advancing this area and several studies with commercial software tools have been described. Agoram et al. (10) showed that the GastroPlus model could be used to explore hypotheses describing the efflux of digoxin by P-glycoprotein expressed in the enterocytes. After optimizing a Michaelis–Menten model of concentration-dependent efflux from the enterocyte to the lumen to match the exsorption of 10% of an intravenous dose, the model was applied to simulate the effects of the P-glycoprotein inducer rifampin on an oral digoxin dose, where a reduction of 41% in the bioavailability was seen because of reduced absorption. In the same paper, a mechanism for the combined effects of P-glycoprotein and intestinal CYP3A4 metabolism on saquinavir absorption was explored. Michaelis–Menten parameters describing the intestinal metabolism were scaled from in vitro data while parameters describing the P-glycoprotein efflux were optimized. Using this model, it was suggested that the increase in bioavailability of an oral dose when taken after a glass of grapefruit juice could be explained by inhibition of both CYP3A4 enzymes and P-glycoprotein. Other workers attempted to model

the influence of P-glycoprotein on the pharmacokinetics of talinolol (63). When using quantitative scaling of in vitro Michaelis–Menten parameters based on experimental data on the distribution in human intestinal tissues (64), they were unable to accurately simulate the in vivo profiles. However, when the model parameters $V_{\max}$ and K_m were optimized, a good description of the nonlinear dose bioavailability relationship was obtained based on a decrease of efflux transport resulting from saturation of P-glycoprotein. The authors hypothesized that the difference between the in vitro value for K_m and the optimized in silico value is due to the way the ACAT model calculates the relevant concentration for interaction with the binding site of P-glycoprotein. They suggested that, as the binding sites for P-glycoprotein lie within the apical membrane of the enterocyte, the much higher intramembrane concentration should be used rather than the cytosolic concentration currently employed. More recently Bolger used GastroPlus to simulate human plasma concentration time profiles expected for simvastatin when administered at a dose of 60 mg with and without grapefruit juice (65). In vitro $V_{\max}$ and K_m for 3A4 metabolism of simvastatin in the gut and liver were reduced 10-fold to account for the inhibitory effect of grapefruit juice. Interestingly, the simulations predicted the unusual effect of increasing fraction absorbed with increasing particle size. Small particles (1 μm) resulted in rapid dissolution and exposure of simvastatin to greater first-pass extraction by CYP3A4 in the proximal small intestine, where CYP3A4 expression is highest. Larger particles (21.5 μm) dissolved more slowly causing absorption to shift to the distal small intestine and colon, and thereby reduced first-pass extraction due to lower expression of CYP3A4 in these regions.

Predicting Variability in Absorption

Interindividual variability in luminal pH, gastric and intestinal motility, solubility, permeability, and mucosal enzymology can result in considerable variability in absorption within a population. Consideration of this variability in simulations is important to ensure that appropriate conclusions are drawn when comparison to observed data is made. Jamei et al. described the application of the ADAM model in SimCYP to predict the variability in fraction absorbed for four diverse drugs. Using the known physiological variabilities on gastric and intestinal transit and volumes, the model was used to simulate variability in fraction absorbed, and produced the expected result that the sensitivity to physiological variability was more pronounced for less permeable compounds. Jamei also simulated the interindividual variability in intestinal drug absorption and metabolism of midazolam (23). The predicted mean first-pass gut extraction (0.40 ± 0.17 SD) was similar to that determined experimentally in anhepatic patients (0.43 ± 0.18 SD), and the observed variability in gut extraction ratio (0.14–0.59) was also predicted well by the simulation. Also using midazolam as a reference drug, Woltosz et al. (66) simulated the variability in plasma levels after an oral dose using the default ACAT model in GastroPlus and the observed expression of CYP 3A4 in liver and gut.

CONCLUSION

Overall, the examples presented in this paper illustrate how absorption models can add value at various stages of the research and development process. For maximum benefit they should be one component in an overall framework

including whole-body physiologically based models for pharmacokinetics and linked to pharmacodynamic models for drug effects. In this way, through formal and realistic integration of data obtained from the various functions supporting project teams, the model acts as a central repository for knowledge and facilitates communication. Of key importance, modeling can assist in translation between preclinical and clinical stages and can contribute to scientifically guided drug discovery and development. Obstacles to the wider use of modeling approaches in the pharmaceutical industry have been attributed to several factors including poorly informed management attitudes, suboptimal organizational structures, lack of adequately trained researchers in modeling, and lack of user-friendly modeling software (67). The commercial software tools described in this chapter are addressing at least the last of these points and considerable progress has been made in recent years. Continued progress is assured if the software companies continue to develop the tools in close collaboration with industry and provide clear and open access to the models basis via adequate documentation and training programs. Industry now needs to recognize the benefits of model-based approaches. Management must adequately resource internal modeling groups and encourage model-based approaches in projects. Scientists must apply the tools systematically and report findings in peer-reviewed journals.

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BACKGROUND

The realization of the importance of product formulation for the speed of onset, intensity, and duration of drug response occurred in the early 1960s. At that time, various scattered reports in the literature (1–5) indicated that formulation changes result in marked differences in maximum observed plasma concentration ($C_{\max}$) and area under the concentration-time curve (AUC), and the term “bioavailability” was coined to describe the fraction of dose reaching the general circulation. A few years later, dramatic bioavailability problems were observed with formulations of phenytoin in Australia and New Zealand in 1968 (6,7) and of digoxin in the United Kingdom and the United States in 1971 (8,9). Consequently, comparative bioavailability studies were introduced in the U.S. regulatory setting (10,11) and the term bioavailability was officially introduced by the Food and Drug Administration (FDA) (12) and defined as follows: “Bioavailability means the rate and extent to which an active drug ingredient or therapeutic moiety is absorbed from a drug product and becomes available at the site of drug action.” Since the late 1970s, a test (T) formulation that meets statistical criteria for the measures of relative bioavailability is termed “bioequivalent” to, and therapeutically interchangeable with, the reference (R) formulation. For more details on the regulatory history of generic drug development, the reader is referred to relevant publications (13–15).

According to the bioavailability definition given above and because of the (most frequently) linear relationship between AUC and the fraction, F , of dose reaching the systemic circulation, AUC is used as a measure of the amount of drug reaching the general circulation.

$$\text{AUC} = \frac{F \times \text{dose}}{\text{CL}} \quad (1)$$

By expressing the parameters of equation (1) in terms of the T and R formulations, assuming that the drug clearance is the same after administration of the two products, that is, $\text{CL}_T = \text{CL}_R$, and assuming that the two formulations contain the same dose that is, $(\text{dose})_T = (\text{dose})_R$, one can easily derive from equation (1) that the ratio of bioavailability coefficients F_T/F_R is equal to the ratio of AUCs.

$$\frac{F_T}{F_R} = \frac{(\text{AUC})_T}{(\text{AUC})_R} \quad (2)$$

Equation (2) comprises the pharmacokinetic (PK) basis for the routine use of AUC in bioequivalence (BE) studies as a robust measure of the amount of drug reaching the general circulation.

The rate of appearance in the general circulation is the second component in the definition of bioavailability. Since most frequently the rate is a

time-dependent parameter, comparisons of rates are based on $C_{\max}$ values because they are supposed to reflect adequately the input rate constant of the drug. Simulations have shown that $C_{\max}$ is not only insensitive to changes in the rate of input, but it is also dependent on amount of drug reaching the general circulation, that is, it is a hybrid parameter (16). Several other absorption rate metrics like $C_{\max}/AUC$ (17), partial area (18,19), and the intercept of $\ln(C/t)$ versus time curve (20) with more favorable kinetic sensitivity properties have been proposed in literature. However, $C_{\max}$ continues to be the unique measure for rate comparisons in the regulatory setting, since it is considered meaningful from a clinical point of view. Today, AUC and $C_{\max}$ are viewed as clinically relevant measures of total and peak exposure, respectively (21).

In the early stages of recognition of BE, physicians by general consensus recommended that a difference of 20% between the two formulations would have no clinical significance for many drugs. This recommendation was interpreted as an allowable difference of 20% in the means of PK variables, namely, AUC and $C_{\max}$, measured after administration of the T and R formulations. However, this definition is a statement for the difference of relative bioavailability of the total production (or population) of the two formulations. Since the assessment of BE relies on a sample of the formulations administered to a limited number of human subjects, regulatory agencies developed methodologies for the assessment of BE on the basis of statistical criteria.

AVERAGE BIOEQUIVALENCE: THE CLASSIC APPROACH

Classically, the assessment of BE relies on the concept of average bioequivalence (ABE) (22). Determination of the ABE of two drug products (T vs. R) is based on the comparison of the means of logarithmically transformed PK parameters, such as AUC and $C_{\max}$. BE is accepted if the difference of the log means (μ_T and μ_R , for the T and the R formulations, respectively) falls between specific pre-defined values for the upper and lower BE limits (23). The current approach of ABE is based on constant BE limits (BEL_0) at a level set by the regulatory agencies (22,24), and usually $BEL_0 = \ln(1.25)$. Thus, the criterion applied for the determination of ABE is

$$-BEL_0 \leq \mu_T - \mu_R \leq BEL_0 \quad (3)$$

In practice, the true population means (μ_T and μ_R) are estimated by the calculated sample averages of the logarithmic parameters of the two formulations (m_T and m_R). In this context, ABE is declared if the calculated 90% confidence interval (CI) for the difference of the log means lies within the preset BE limits (23). Assuming the classic two-treatment, two-period, crossover BE study design, with equal numbers of subjects in each sequence, the upper and lower limits of the 90% CI are calculated according to equation (4).

$$(\text{Upper, Lower limit of the 90\% CI}) = \exp\left(\text{Diff} \pm t_{0.05, N-2} \sqrt{\frac{s^2 2}{N}}\right) \quad (4)$$

where Diff is the difference of T and R means, that is, $\text{Diff} = m_T - m_R$; t , the student's statistic; s^2 , the residual variance, calculated by the residual mean square error of analysis of variance (ANOVA)—reflecting within-subject variance; and N , the number of subjects. The usual statistical approach for

the evaluation of ABE consists of two one-sided t procedures (25) to determine if the PK measures of the T and R products are comparable. This definition of ABE ensures the consumer safety, since the probability of an erroneous acceptance of BE does not exceed the preset level of significance (22).

During the past three decades or so, significant contributions to the theoretical and practical aspects of BE have been made by professional associations such as the American Pharmaceutical Association, American Association of Pharmaceutical Scientists, and regulatory bodies, for example, FDA and European Medicines Evaluation Agency (EMA). Also, international symposia (26–28) have contributed to the evolution of BE studies and methodology. During the last decade, significant advances on scientific issues relating to BE assessment have been made in three areas: in the assessment of the BE of highly variable (HV) drugs and drug products (28), in identifying situations where BE could or should be based on the plasma levels of metabolites (28), and in identifying situations where an *in vivo* BE study could be waived (29). In this chapter, developments in the first two areas will be presented, as advances in the third area are presented in detail in chapter 18 of this book.

HIGHLY VARIABLE DRUGS AND DRUG PRODUCTS

The Problem in Establishing BE

A drug or drug product is usually characterized as HV if the within-subject coefficient of variation (CV) of its PK responses is $\geq 30\%$ (30–35). It is worth mentioning that the CV is related to the residual variance s^2 in the log scale, calculated by ANOVA, with the formula: $s^2 = \ln(CV^2 + 1)$. For HV drugs, the 0.80 to 1.25 BE limits seem to be too restrictive, leading to high producer risks (30–32,36,37).

As can be seen from equation (4), the width of the 90% CI is proportional to the within-subject variability and inversely proportional to the number of subjects participating in the study. Consequently, as within-subject variability increases, a higher rejection rate of BE for truly equivalent drug products is observed. Therefore, for truly equivalent products, it becomes too difficult to establish BE unless a large number of subjects are recruited to achieve adequate statistical power.

The need for unusually large numbers of healthy volunteers for the assessment of BE of HV drugs can raise ethical and practical issues. The exposure of large numbers of healthy volunteers to a drug even if it is deemed to be “safe,” to satisfy a traditional preset criterion, must be seriously considered (38). In addition, the increase in the cost of the investigations of HV drugs—with usually wide therapeutic indices (35,38)—may result in difficulties in the development of new or generic drug products.

In the case where the upper limit of the 90% CI (equation 4) falls exactly on the upper preset BE limit, Diff becomes equal to $\text{Diff}_{\max}$, which is the maximum acceptable difference between means (25,39). $\text{Diff}_{\max}$, and therefore the maximum acceptable geometric mean ratio ($\text{GMR}_{\max}$) [$\text{GMR}_{\max} = \exp(\text{Diff}_{\max})$], for a given number of subjects, is related to not only the estimated intrasubject variance but also the value of the preset upper BE limit.

The major feature of the definition of classic unscaled ABE relies on the fact that two constant “borderline” values (0.80 and 1.25) are assigned for BE limits. Under this condition, extreme geometric mean ratio (GMR) values, which ensure

BE, converge at unity as intrasubject variability increases (25,40). In other words, when upper and lower BE limits are fixed, the demonstration of BE requires that the means of two products must be as close as possible as variability increases. Although setting constant the BE limits is conceptually fundamental, the 0.80 to 1.25 limits appear very "strict" in the case of HV drugs. To overcome the difficulties encountered in the assessment of the bioavailability of HV drugs with the currently used BE limits, several approaches have been proposed.

Approaches for the Evaluation of BE

Multiple-Dose Studies

To reduce within-subject variability, multiple-dose steady-state studies have been considered (24,41). It has been shown that the observed variation of PK parameters is often lower at steady state than after single dosing (41–43). The reduced variation of $C_{\max}$ at steady state is probably due to its lower kinetic sensitivity in reflecting absorption rate (22,44). Nevertheless, under certain conditions, $C_{\max}$ was found to exhibit higher variation at steady state than after a single administration, and therefore multiple-dose designs were not considered to be the solution for the assessment of BE of HV drugs (43,45). Currently, the FDA approach recommends applicants to conduct single-dose studies rather than multiple-dose studies because "single-dose studies are generally more sensitive in assessing release of the drug substance from the drug product into the systemic circulation" (22).

Replicate Designs

For single-dose studies, replicate designs that reduce the total number of subjects required have been also proposed (22,24,32,41) for the assessment of BE for HV drugs. Roughly, about half as many volunteers are needed in a four-period study than in a two-period investigation to attain the same statistical power.

However, replicate designs (as multiple-dose studies) lead to increased duration of exposure to the drug and, moreover, potential practical problems may arise, for example, increased incidence of subject withdrawals. In addition, in certain cases, for example, for drugs with long half-lives, replicated designs are difficult to apply.

Consideration of Only the Point Estimate of the Mean

Test/Reference Ratio

Another approach for the assessment of BE of HV products has been based on the point estimate of the mean T/R ratio. In this context, a relaxed requirement is adopted by the regulatory authority in Canada in the case of $C_{\max}$. This PK parameter is a single-measure estimate and often shows higher variation than AUC. Therefore, Health Canada requires that only the point estimate of the GMR for $C_{\max}$, and not its 90% CI, fall between the BE limits of 0.80 to 1.25 (46).

Individual BE

Individual BE (23,47–52), a procedure relying on the concept of switchability between drug formulations, has been also proposed for the evaluation of HV drugs. According to this concept, the T-R difference is compared with the R-R difference within subjects using repeated measures. The individual BE criterion comprises the ratio of the sum of the contrast of the squared means of the two

formulations, the contrast of their within-subject variances and the subject by formulation variance over the within-subject variance of the R formulation. While individual BE represents an attractive approach, several problems have limited its application in practice (38,51).

Widening of Acceptance Limits to Prefixed Constant Values

Widening the BE acceptance limits to prefixed constant values (0.70–1.43 or 0.75–1.33) (24,31,53) has been proposed, especially for PK parameters showing increased variation, for example, $C_{\max}$.

Expanded 0.75 to 1.33 or 0.70 to 1.43 BE limits for drugs meeting a “high-variability criterion.” Several questions may arise, indicative of the difficulties of the application of this approach: What is the high variability criterion? An intra-subject variability value, estimated from ANOVA? For example, when $CV > 30\%$ (33,34)? A problem may arise about the classification of drugs presenting borderline variability values (54). It was estimated that about 20% of the evaluated HV drugs constitute borderline cases (34). Nevertheless, the use of an extended region of acceptance reduces the producer risk at high CV values, but at the same time large differences between the means are allowed (55) for drug products with moderate residual variability. This constitutes a potential problem of switchability for multisource formulations, each declared bioequivalent to the same R product (39,56). Consequently, an additional point estimate constraint criterion on GMR, for example, $0.80 \leq GMR \leq 1.25$, may be needed.

Widening of BE limits only beyond a limiting, “switching” variability value (mixed model). It has been suggested to use either the classic 0.80 to 1.25, or the more “liberal” (e.g., 0.75–1.33 or 0.70–1.43) BE limits only beyond a switching variability value (24,53). However, apart from the fact that in this case two criteria are required, applying an arbitrarily chosen switching variability value can lead to unfair treatment of different formulations of the same drug evaluated in separate BE studies and presenting only minor differences in variability (57). For example, assuming a switching variability of $CV = 30\%$, it seems rather unfair that a drug with broad therapeutic index and $CV = 29.9\%$ has to be evaluated using the classic 0.80 to 1.25 BE range, which allows a maximum accepted value of GMR, $GMR_{\max} = 1.08$, while the same drug could be evaluated in a 30%, using the expanded 0.70 to 1.43 range, which allows a $GMR_{\max} = 1.24$ (see Fig. 6B of Ref. 57). The major cause of this attribute is the inherent discontinuity when these two BE criteria are concomitantly applied (Fig. 1). Consequently, a question arises: How do we deal with BE studies with borderline variability values, that is, BE trials presenting variability values very close to the switching variability?

Scaled Procedures

A method for expanding the limits for HV drugs, based on an estimate of intrasubject variation, was proposed: The BE limits are scaled according to a fixed multiple of within-subject standard deviation, σ_w , on the log scale (58).

$$(\text{Upper, Lower BE limit}) = \exp(\pm k\sigma_w) \quad (5)$$

where k is a multiplying factor.

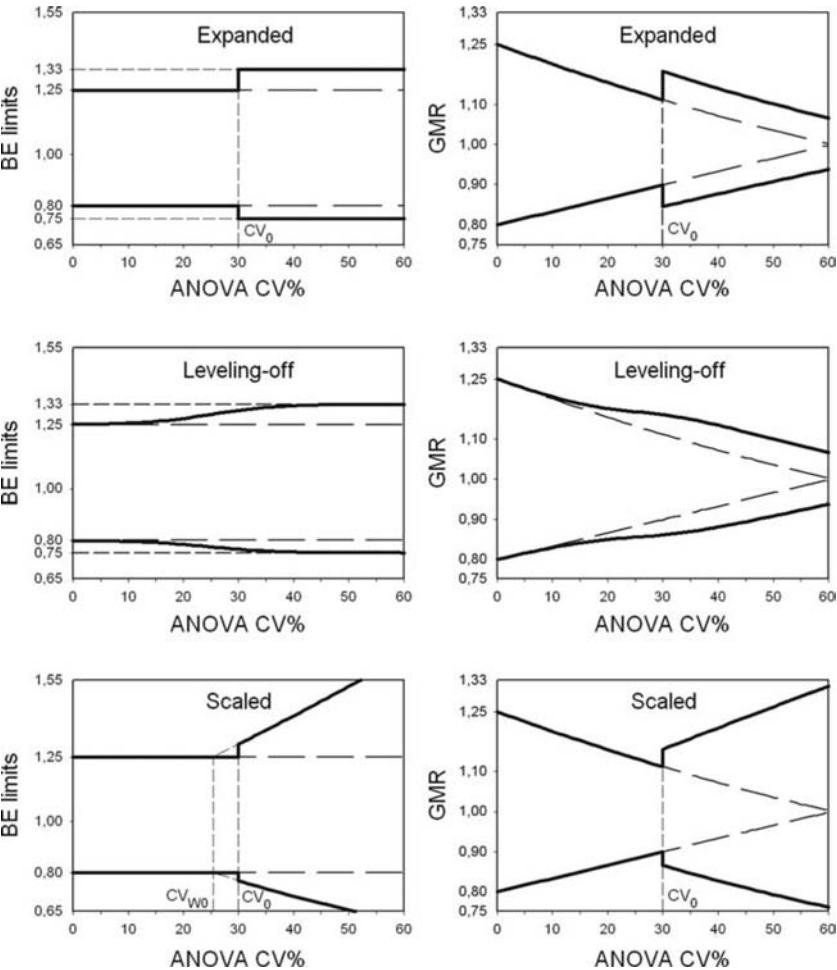


FIGURE 1 BE limits (*left side*) and extreme GMR values, which ensure BE (*right side*) as a function of within-subject variability (ANOVA CV), for the classic (0.80–1.25) limits (*dashed lines*) and three proposed procedures (*solid lines*): expanded BE limits beyond a switching variability $CV_0 = 30\%$ (24) (*top*); BE limits with leveling-off properties based on a sigmoid function (63) (*middle*); and scaled BE limits (equation 10) with a preset variability $CV_{W0} = 25.4\%$ and switching variability $CV_0 = 30\%$ (35,64) (*bottom*). A two-period crossover study with 36 subjects was assumed for the calculation of extreme GMR values. *Abbreviations:* BE, bioequivalence; GMR, geometric mean ratio; CV, coefficient of variation.

Thus, the acceptance criterion can be expressed as

$$-k\sigma_W \leq \mu_T - \mu_R \leq k\sigma_W \tag{6}$$

It has been also suggested that the regulatory criterion of ABE, in the case of HV drugs, could be scaled by a standard deviation, leading to an approach known

as scaled average bioequivalence (ABEsc) (59,60). The acceptance criterion is then defined as

$$-k \leq \frac{\mu_T - \mu_R}{\sigma_W} \leq k \quad (7)$$

The scaling factor, σ_W , in the case of a two-period design is the residual standard deviation, σ_{Res} , estimated from ANOVA, while for a replicate design the within-subject standard deviation of the R formulation, σ_{WR} , is used.

An approach using the noncentral t distribution to calculate the confidence limits for ABEsc has been suggested (60). An alternative procedure consisting of a numerical approximation based on the method of Hyslop et al. (61) has been also proposed for the statistical evaluation of ABEsc.

It is worth mentioning that the model for ABEsc (equation 7) can be readily converted to that of the scaled BE limits (equation 6). Indeed, when investigated, the two approaches yielded very similar results (60).

Various suggestions have been made for the most appropriate proportionality factor, k , for scaled BE limits (39,58,62). The value of k affects the slope of the BE limits and therefore the degree of expansion.

Simple scaled BE limits. When variability is low, very small deviations of GMR from unity are permitted to declare BE. Consequently, scaled BE limits appear to be very strict for drugs with low variability and probably inappropriate even for the evaluation of drugs with a narrow therapeutic range. At a specific value of the variability ($\sigma_W = \sigma_0$), depending on the value of the proportionality factor k , scaled BE limits become equal to the classic BEL_0 .

$$k\sigma_0 = BEL_0 = \ln(1.25) \quad (8)$$

As variability increases, scaled BE limits become very liberal, allowing GMR values higher than 1.25 (40). Therefore, a common drawback of the reported scaled BE limits (39,58,62) is their continuous increase with variability. This leads to very broad acceptance limits of BE. The GMR acceptance region has a nonconvex shape (40), similar to that for the Hauck and Anderson procedure as pointed out by Schuirmann (see Fig. 12 of Ref. 25), and gets wider and wider with increasing CV. Thus, BE studies with GMR deviating considerably from unity even at very high CVs could be accepted. Since large differences between the means can be accepted by scaled methods with substantial probabilities, an additional regulatory criterion was proposed to be imposed concomitantly with the CI test (53). This secondary criterion suggests that the estimated GMR should be constrained in the range 0.80 to 1.25. Nevertheless, even with the concomitant application of the above-mentioned additional criterion, the acceptance region still has a nonconvex shape, and BE studies with GMR values between 0.80 and 1.25 can be accepted, even at very high variability level.

Mixed model. An interesting variant of the simple scaled procedure has been proposed (62). It involves the use of both the classic unscaled ABE (when drugs do not exhibit high variability) and the ABEsc for HV drugs (when a preset magnitude of the variability is exceeded) (62). The switching variability, σ_0 , for the ABEsc was set to 0.20, and corresponds to a proportionality constant, $k = \ln(1.25)/\sigma_0 = 1.116$. This mixed model (62) for ABEsc can be converted to a

mixed approach of scaled BE limits, using the classic unscaled criterion up to CV 20% and scaled BE limits with a proportionality factor of 1.116, for CV over 20%. When the mixed model is used, the boundaries of the GMR acceptance region converge to a minimum value as CV values increase up to 20% and then start to spread apart for values of CV higher than 20% (see Fig. 2 of Ref. 40). Consequently, this approach is less “permissive” for drugs with moderate variability (CV ~ 20%) than for drugs with low or high variability. The nonmonotony of the extreme accepted GMR versus CV plots is an unfavorable property of the method because it appears to “punish” drug products with moderate variability (40). Moreover, as the mixed model is a scaled procedure, it suffers also from the common drawback of the simple scaled BE limits mentioned previously, that is, the continuous increase with variability leading to very broad acceptance BE limits. Again, the GMR acceptance region has a nonconvex shape and an additional (3rd) point estimate constraint criterion, for example, $0.80 \leq \text{GMR} \leq 1.25$ may be needed. Nevertheless, BE studies with GMR deviating from unity can be accepted even at very high CVs.

Finally, if one uses a different value for k , for example, $k = 0.760$ (39,62) for the mixed model, the switching variability, σ_0 , is 0.294 (corresponding to a CV = 30%), and a stricter BE criterion is constructed.

Combined scaled criterion. To improve the performance of the above-mentioned scaled procedures, a novel approach has been proposed consisting of a combined criterion for evaluating BE (40). Scaled BE limits containing an effective constraint have been developed. The proposed BE limits scale with intrasubject variability but incorporate a GMR-dependent criterion too, which makes them less permissive as GMR values depart from unity (40,57).

Scaled BE Limits with Leveling-off Properties

A new rationale for the design of scaled BE limits has been developed (63) to improve the excessively restrictive behavior of the classic BE limits when truly bioequivalent HV drugs are compared, and concomitantly to avoid the drawbacks of the simple scaled or mixed methods, discussed previously. To this end, the BE limits developed scale with intrasubject variability but only until a “plateau” value and combine the classic (0.80–1.25) and expanded (0.75–1.33) BE limits into a single criterion (Fig. 1). To combine the above-mentioned desired properties into a single criterion, the upper BE limit is expressed as a function of intrasubject variability, which levels off at a predefined plateau value. Accordingly, this function has three controlling parameters, which are

1. the minimum (or starting) value of the upper BE limit,
2. the maximum (or plateau) value of the upper BE limit, and
3. the “rate” of the gradual change of the upper BE limit value as a function of variability.

The new scaled limits become more permissive than the classic unscaled BE limits as variability increases, and thus they require fewer subjects to prove BE. Nevertheless, the GMR acceptance region has a convex shape (Fig. 1), which is similar to that of the classic unscaled 0.80 to 1.25 limits (29,40). Undoubtedly, this is not only a desired property but also a unique characteristic for a scaled

method. This finding is a consequence of the new structure of the BE limits with leveling-off properties.

One of the major advantages of the new scaled limits is their gradual expansion with variability until a plateau value is reached. The gradual expansion of the BE limits is by far preferable than the use of expanded criteria only beyond an arbitrarily chosen, critical switching variability value (Fig. 1), as the discontinuity of the BE limits may lead to preferential treatment of drugs presenting only minor differences in variability. The gradual expansion from a strict to a permissive BE limit, apart from avoiding the discontinuity around a switching variability, makes the new BE limits also suitable for use at low CV levels. In fact, when variability is low, BE limits with leveling-off properties exhibit similar percentage of accepted BE studies as the classic BE limits (63). Therefore, these BE limits would be implemented in practice, for example, in the case of $C_{\max}$ ratio, in lieu of a wider acceptance interval (23). It is also worthy to mention that leveling-off BE limits present a quite flexible structure, and therefore a variety of starting and plateau values for the upper BE limit can be considered. The flexibility, continuity, and leveling-off properties of these scaled BE limits in conjunction with their performance in simulation studies (63) make them suitable for the assessment of BE studies without the need of a secondary criterion of constrained GMR value and irrespective of the level of variability encountered.

Current Thinking Within the FDA for the Evaluation of HV Drugs and Drug Products

For drugs with an expected within-subject variability of $\geq 30\%$, a BE study with three-period, R-replicated, crossover design has been proposed (34,35,64). The minimum number of subjects that would be acceptable is 24. The BE assessment comprises two parts: an ABESc evaluation and a point estimate constraint. The BE criterion for both AUC and $C_{\max}$ is defined as

$$\frac{(\mu_T - \mu_R)^2}{\sigma_{WR}^2} \leq \theta \quad (9)$$

where $\theta = (\ln \Delta)^2 = \sigma_{W0}^2$, with $\Delta = 1.25$ and $\sigma_{W0} = 0.25$ (the preset standard variability).

A 95% upper confidence bound for $(\mu_T - \mu_R)^2 / \sigma_{WR}^2$ must be $\leq \theta$, or equivalently a 95% upper confidence bound for $(\mu_T - \mu_R)^2 - \theta \sigma_{WR}^2$ must be ≤ 0 . Additionally, the point estimate for GMR of T/R must fall within 0.80 to 1.25.

In the original scale, the proposed BE limits are

$$(\text{Upper, Lower BE limit}) = \exp\left(\pm \frac{\ln(1.25)}{0.25} \sigma_{WR}\right) \quad (10)$$

According to this criterion, the value of the k factor chosen is $k = \ln(1.25)/0.25 = 0.892$, presenting an intermediate value between the too liberal approach of $k = 1.116$ (62) and the stricter one, $k = 0.760$ (39,62). However, the choice of this value (or equivalently the choice of $\sigma_{W0} = 0.25$) presents the demerit of an inherent discontinuity of the BE limits when applied for drugs with $CV \geq 30\%$ (i.e., with $\sigma_{WR}^2 \geq 0.294$) (Fig. 1). The cause of this attribute is that the preset standard variability value ($\sigma_{W0} = 0.25$) is not the same as the switching

variability value ($\sigma_0 = 0.294$). A relevant comment has also been made recently (65). Consequently, if the estimated within-subject CV of the R formulation is just above the changeover point of 30%, the BE limits will be much wider (i.e., >1.30) than just below (i.e., 1.25).

Moreover, the proposed procedure suffers from the same drawbacks as all the mixed models of the scaled methods: The boundaries of the GMR acceptance region converge to a minimum at the switching variability value and then start to spread apart for higher values of CV, presenting a nonconvex shape (Fig. 1). Consequently, an *additional* point estimate constraint criterion on GMR is needed.

The EMEA Approach for the Evaluation of HV Drugs

EMA in the Note for Guidance on the Investigation of Bioavailability and Bioequivalence (24) states that the 90% CI for AUC and $C_{\max}$ ratios should lie within an acceptance interval of 0.80 to 1.25. However, "in certain cases a wider interval may be acceptable" for $C_{\max}$ (Fig. 1), provided that there are no safety or efficacy concerns. Some points of this statement were furthermore clarified in a Questions & Answers document (33) as follows: The possibility offered by the guideline to widen the acceptance range "should be considered *exceptional* and *limited to a small widening* (0.75–1.33)." Furthermore, this possibility is restricted to those products for which at least one of the following applies: Safety and efficacy should be *clinically justified* [i.e., using adequate pharmacokinetic/pharmacodynamic (PK/PD) or clinical data], or should refer to a *defined HV drug* (i.e., an R product with intrasubject variability greater than 30%). Recently, EMA has addressed more intensively the issue of HV drugs. In this context, the Committee for Medicinal Products for Human Use (CHMP) has also released a concept paper for an addendum focusing on scaled procedures for the evaluation of BE of HV drugs (66) and a recommendation document on the need for revision of the note for guidance (67).

METABOLITES IN BIOEQUIVALENCE ASSESSMENT

In the majority of cases, assessment of BE relies on the plasma concentrations of the parent drug since either this is the only reported therapeutic moiety or it is not metabolized. Concern is raised, however, when the parent drug is metabolized and the metabolite(s) exhibit comparable therapeutic activity with the parent drug. On the other hand, obvious reasons for measuring the metabolite(s) are (i) whenever an inactive prodrug is metabolized to an active metabolite and (ii) the parent drug concentrations are too low while metabolite(s) plasma levels are quantifiable. The reader can find several examples in the literature, whereas the target species for measurement is either the metabolite(s) or the parent drug and the metabolite(s) (68–76).

Computer-simulated BE studies are a powerful tool in this field of research since the modeling assumptions along with the values of the parameters are specified and the results can be contrasted with the assumptions used. The simulations are based on classical PK models with the formation of metabolite taking place during the presystemic absorption and/or during subsequent recirculation through the liver. The simulations try to explore which of the species is the most appropriate for BE decision making on the basis of statistical criteria such as the width of the relevant CIs. One should

recall, however, that all these approaches are approximations of the reality because the complexity variability in hepatic clearance can also be a function of the magnitude of alternative elimination processes for the drug and/or the metabolite. During the last 15 years or so, several simulation studies on the role of metabolites in BE have been published (77–82). Many of these studies have been reviewed by Midha and colleagues (83), and the use of metabolites in BE studies has been the subject of a recent Bio-International congress (28).

The first study (77) in this topic published in 1991 was based on a simple first-order one-compartment PK model, with exclusive formation of a metabolite during recirculation through the liver. The authors focused on the rate of metabolite elimination being limited by either its formation or its excretion. Simulated BE studies were carried out with random error added to the absorption rate constant values of the R and T formulation. The statistical analysis based on the comparison of variability (using 90% CIs) associated with the $C_{\max}$ values of parent drug and the metabolite revealed that the former was greater than the latter. Although their simulation results were contrasted with experimental BE studies of four drugs, caution should be exercised whether the drugs fulfill the modeling assumptions relevant to the metabolism of drug (83).

The second study by the same authors four years later (78) utilized a two-compartment model, with formation of the metabolite taking place either pre-systemically or during recirculation through the liver. Again, comparisons were based on the variability of $C_{\max}$ values for the parent drug and the metabolite as a function of the variabilities used for the absorption rate constant of the parent drug, k_a , as well as the first-pass formation of the metabolite, k_f . The variability of $C_{\max}$ values of the parent drug and the metabolite was found to follow the magnitude of variability associated with k_a and k_f , respectively.

The work of Tucker and colleagues (79) has been based on a model in which the formation of metabolite in the liver takes place both on first passage and on subsequent recirculation through the organ. The analysis was focused on AUC values derived from simulation studies of drug and metabolite kinetics. The PK parameters considered were intrinsic, CL_{int} and renal clearance, CL_r as well as the hepatic blood flow, Q_H . According to the authors, metabolite data have to be used for high-extraction-ratio drugs, namely, $CL_{\text{int}} \geq Q_H$. For low extraction ratio drugs ($CL_{\text{int}} < Q_H$), the parent drug data are preferred; however, when CL_r is low, one has to use metabolite data. The basic conclusion of the study is that the within-subject variabilities of metabolic and renal clearances are the basic determinants for the use of drug or metabolite data since they determine the sensitivity of AUC to the differences of fraction of dose reaching the general circulation.

In similar work, Rosenbaum and Lam (80) studied the sensitivities of the parameters AUC and $C_{\max}$ of the parent drug and the metabolite to variabilities associated with the intrinsic and hepatic clearance. A simple PK model was utilized with the formation of a single metabolite taking place during first passage. The statistical analysis of data revealed that the parent drug had wider 90% CIs around the point estimates for the ratio (T/R) of geometric means of AUC and $C_{\max}$ than the corresponding one for the single metabolite. In a similar vein, Rosenbaum (81) used a semiphysiological pharmacostatistical model to study the manner in which intraindividual variability in hepatic clearance is transferred to AUC of a drug and its metabolite. The model assumes the formation of metabolite in the liver both on first passage and on subsequent recirculation through the organ. The results indicated that as the drug's hepatic

extraction ratio increased, the variability of the drug's AUC was increased, whereas that of the metabolite decreased.

Jackson (82) carried out simulations, focusing on the response of parent drug and metabolite 90% CIs for AUC and $C_{\max}$ to equivalent and inequivalent immediate-release formulations. A linear first-pass model with random error added to the model parameters: renal clearance, hepatic clearance, systemic clearance, and liver blood flow. Specific values were assigned to the absorption rate constant and fraction absorbed to investigate problems associated with equivalent and nonequivalent immediate-release formulations. According to Jackson (82), the $C_{\max}$ for the parent drug provided the most accurate assessment of BE. On the contrary, the metabolite $C_{\max}$ was found to be insensitive to changes related to rate of absorption. In addition, when the value of the intrinsic clearance is higher than the liver blood flow, the use of the metabolite $C_{\max}$ data can lead to a conclusion of BE for truly bioinequivalent products.

In parallel, the use of prodrugs in therapy is pertinent to the matter since most of them are rapidly absorbed from the gastrointestinal tract and rapidly biotransformed to the active metabolite. Prodrug blood levels tend to be very low and much more variable when compared with the active metabolite. It should be noted that many prodrugs (angiotensin converting enzyme inhibitors, some statins, valacyclovir, fenofibrate) were not quantified with analytical methods of high sensitivity in PK studies by the innovator because of their short residence time and low blood levels. However, the continuous evolution in mass spectrometry allows today for the reliable measurement of prodrugs for a reasonable period of time. Thus, the measurement of both the prodrug and the active metabolite for the assessment of BE remains to be further evaluated. To emphasize the contradictory approaches as well as the incoherence of the description of the current guidelines (22,24) for the role of metabolites in BE assessment, we quote below two characteristic extracts. The FDA guideline (22) states,

The moieties to be measured in biological fluids collected in bioavailability and bioequivalence studies are either the active drug ingredient or its active moiety in the administered dosage form (parent drug) and, when appropriate its active metabolite. . . . Measurement of a metabolite may be preferred when parent drug levels are too low to allow reliable analytical measurement in blood, plasma or serum for an adequate length of time. . . . If the metabolite contributes meaningfully to safety and/or efficacy, we also recommend that the metabolite and the parent drug be measured.

The EMEA guideline (24) states,

In most cases evaluation of bioavailability and bioequivalence will be based upon the measured concentrations of the parent compound. In some situations, however, measurements of an active or inactive metabolite may be necessary instead of the parent compound. . . . Bioequivalence determinations based on metabolites should be justified in each case bearing in mind that the aim of a bioequivalence study is intended to compare the in vivo performance of T and R products. In particular if metabolites significantly contribute to the net activity of an active substance and the pharmacokinetic system is nonlinear, it is necessary to measure both parent drug and active metabolite plasma concentrations and evaluate them separately.

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Biowaiving Based on the BCS— A Global Comparison

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INTRODUCTION

Introduction of the Biopharmaceutics Classification System (BCS) (see chap. 8) was intended to reduce in vivo bioequivalence studies, in particular for applications of generic drug products. Actually, in its simplifications, the BCS concept addresses the complex question of what affects drug substance bioavailability and when formulation effects may be considered negligible or may even be supposed to be absent. This mechanistic view of bioavailability ultimately allowed implementation of the BCS-based biowaiver into guidance documents on bioequivalence, since the bioequivalence of oral dosage forms is the primary issue for which the BCS concept can be applied in a regulatory setting.

The conceptual background and basic requirements of the BCS-based biowaiver are well appreciated by applicants and regulatory assessors in a general sense. However, the choice of specific requirements to achieve satisfactory experimental and/or other supportive data in the regulatory context seems to be less obvious. This may at least in part be due to the fact that regulatory assessment of applications is generally separated into quality and clinical (toxicological) sections. Whereas the bioequivalence data which are used to demonstrate equivalent safety and efficacy of different formulations are usually part of the clinical assessment, BCS-based biowaiver submissions may be assessed by regulators that are more used to reviewing quality data. For these reasons, implementation of the concept is not yet widespread and in fact has been realized in only a very limited number of jurisdictions to date.

In addition, requirements differ between guidance documents that have implemented the BCS-based biowaiver as a means to prove bioequivalence. This may at least in part be attributed to the different time periods over which the guidelines were developed and hence available practical knowledge at that time. Another reason is the increasing interest in the BCS approach and scientific discussions and findings based on growing data sets during the last couple of years. Ongoing discussions on possible relaxation of the initially rather conservative criteria to be met for BCS-based biowaiver applications may account for certain differences between early and recent regulatory requirements. This chapter gives an overview on the current status, highlighting the main guidance documents with their similarities and differences.

THE U.S. FDA GUIDANCE

The U.S. FDA was the first jurisdiction that implemented regulatory requirements for BCS-based biowaiver applications in a separate, comprehensive guidance document in 2000 (1). This guidance document is closely related to the basic BCS concept initially introduced and published by Amidon et al. (2). The guidance restricts the eligibility of the BCS-based biowaiver approach to

BCS class I drug substances in immediate-release formulations and requires in vitro dissolution to be rapid, that is, at least 85% dissolution of the labeled amount within 30 minutes or less. The underlying scientific rationale is explained as follows: “the rate and extent of drug absorption is assumed not to be dependent on product formulation as long as the drug substance is highly soluble and easily transported and is manufactured in immediate-release dosage forms exhibiting similar, rapid in vitro dissolution characteristics. Performing in vivo bioequivalence studies is considered unnecessary under these circumstances provided excipients used in the formulations are similar and/or are not expected to differ in their impact on absorption processes.” (1)

According to the U.S. FDA guidance, BCS-based biowaivers are not acceptable for narrow therapeutic range drugs irrespective of their BCS classification. Illustrative examples are mentioned like, for example, digoxin, lithium, phenytoin, theophylline, and warfarin. The reader may also refer to the SUPAC (Scale-Up and Post-Approval Changes) guidance (3) from 1995, which gives a more comprehensive table of drugs and formulations considered to be narrow therapeutic index drugs (Table 1). It should be noted, however, that some modified-release formulations are also included in this listing, which of course are not eligible for a BCS-based biowaiver. Products designed to be absorbed in the oral cavity (e.g., sublingual or buccal tablets) are also excluded from the BCS-based biowaiver concept. The latter exclusion is deemed obvious since absorption may already start immediately after administration through the oral mucosa, thus conceptual prerequisites do not meet the intended performance properties of such products.

TABLE 1 Narrow Therapeutic Range Drugs According to the U.S. FDA

Aminophylline tablets, ER tablets
Carbamazepine tablets, oral suspension
Clindamycin Hydrochloride capsules
Clonidine Hydrochloride tablets
Clonidine Transdermal Patches
Dyphylline tablets
Disopyramide Phosphate capsules, ER capsules
Ethinyl Estradiol/Progestin oral contraceptive tablets
Guanethidine Sulfate tablets
Isoetharine Mesylate Inhalation Aerosol
Isoproterenol Sulfate tablets
Lithium Carbonate capsules, tablets, ER tablets
Metaproterenol Sulfate tablets
Minoxidil tablets
Oxtriphylline tablets, DR tablets, ER tablets
Phenytoin Sodium capsules (prompt or extended), oral suspension
Prazosin Hydrochloride capsules
Primidone tablets, oral suspension
Procainamide Hydrochloride capsules, tablets, ER tablets
Quinidine Sulfate capsules, tablets, ER tablets
Quinidine Gluconate tablets, ER tablets
Theophylline capsules, ER capsules, tablets, ER tablets
Valproic Acid capsules, syrup
Divalproex Sodium DR capsules, DR tablets
Warfarin Sodium tablets

Abbreviations: ER, extended release; DR, delayed release. Source: From Ref. 3.

According to the U.S. FDA guidance, BCS-based biowaivers are applicable to bioequivalence considerations for products containing BCS class I drugs to be addressed in the framework of Investigational New Drug (IND) applications, New Drug Applications (NDAs), Abbreviated New Drug Applications (ANDAs), and postapproval changes. It is specifically mentioned that the BCS approach can be used to justify the absence of *in vivo* bioequivalence studies, but not for other types of bioavailability or pharmacokinetic studies.

The solubility and permeability class boundaries, dissolution requirements, as well as formulation considerations to support a BCS-based biowaiver are outlined in the following.

Solubility

The definition of “high solubility” refers to the highest dose strength of an immediate-release product, which has to be soluble in 250 mL or less of aqueous media over the pH range of 1 to 7.5, a range that is considered to be physiologically relevant. Solubility measurements should be performed at 37°C using a stability-indicating, validated method.

Experimental requirements like number of replicates, consideration of the pK_a , and stability issues are extensively outlined and are basically in line with pharmacopoeial recommendations.

Permeability

The classification regarding high permeability refers to the extent of absorption in humans. Accordingly, a drug substance is considered “highly permeable” if the extent of absorption in humans reaches at least 90% of an orally administered dose. To prove that a drug substance is highly permeable, the following experimental methods are mentioned as acceptable in the BCS framework:

1. Pharmacokinetic studies in humans
 - Mass balance studies (stability considerations should be noted)
 - Absolute bioavailability studies
2. Intestinal permeability methods
 - *In vivo* intestinal perfusion studies in humans
 - *In vivo* or *in situ* intestinal perfusion studies using suitable animal models
 - *In vitro* permeation studies using excised human or animal intestinal tissues
 - *In vitro* permeation studies across a monolayer of cultured epithelial cells

Suitability of any chosen method must be demonstrated. It is stated that *in vivo* or *in situ* animal models and *in vitro* methods are considered appropriate for passively transported drugs, and the use of internal standards is recommended to facilitate correct permeability classification. A specific list is given as an attachment A to the guidance document, identifying model drugs as potential internal standards to be used for intestinal permeability experiments.

In Vitro Dissolution

Comparative *in vitro* dissolution investigations should ensure similar rapid dissolution of the active pharmaceutical ingredient (API) from the test and reference product within the stated pH range. Accordingly, no less than 85% of

the labeled amount should be dissolved within 30 minutes in each of the required media: 0.1 N HCl, pH 4.5, and 6.8 buffers. Regarding experimental requirements, reference is made to the U.S. Pharmacopoeia and the U.S. FDA Guidance for Industry on Dissolution Testing of Immediate-Release Solid Oral Dosage Forms (August 1997) (4). A minimum of 12 dosage units of the test and reference product should be investigated and the resulting profiles compared using the similarity factor (f_2), unless 85% or more of the labeled amount dissolves within 15 minutes from both products. The latter case would allow the conclusion that the investigated products are similar without further statistical calculations.

Formulation Considerations

Since excipients may differ considerably between a generic and an innovator product, it has to be ensured that those differences will not affect rate and extent of absorption. In addition, it may be possible that particular excipient-driven effects may not be detectable by means of *in vitro* dissolution experiments. Therefore, the U.S. FDA guidance requires that excipients be employed in usual quantities and be consistent with their intended function. New excipients and/or atypically large amounts of commonly used excipients require additional information and discussion. It is stated that a study on relative bioavailability (i.e., using a simple aqueous solution as a reference) may be necessary to prove that certain excipients are not likely to have an impact on bioavailability.

In its last section, the U.S. FDA guidance outlines detailed recommendations regarding the filing of a BCS-based biowaiver for the regulatory authority.

Current Status

Although the U.S. FDA guidance has been in place since the year 2000, BCS-based biowaivers have been granted for less than 20 drug substances (personal communication, U.S. FDA) up to now. Hence, the number of BCS-based biowaiver applications still remains limited. The possibility of revising the guidance document has been addressed with a view to making the biowaiver-based approval mechanism more accessible, a revision that might include some modifications of class boundaries. However, as of this writing, no changes have been instigated.

THE EUROPEAN GUIDANCE

The BCS-based biowaiver has been also implemented in the European note for guidance (NfG) on bioequivalence testing that came into operation in 2002 (5). During the preparation of this manuscript, the guidance was revised and a new draft guidance issued (see later in the text and Ref. 9), but since it is still open for comment and has not yet been officially adopted, the 2002 guidance will be addressed here first.

In contrast to the comprehensive U.S. FDA recommendations, the BCS concept is currently addressed only briefly on one page of the NfG. Generally, reference is made to the BCS but only limited details are given.

Similar to the U.S. FDA guidance, therapeutic aspects are addressed first, that is, the drug substance in question should be “uncritical” in terms of bioequivalence and possible therapeutic failures—this requirement may be

interpreted as needing to possess an uncritical (wide) therapeutic range. This recommendation is meant to serve as the initial risk assessment to justify waiving in vivo bioequivalence testing for a particular drug substance.

Class boundaries and formulation-related requirements are addressed in the following sections.

Solubility

The criterion for high solubility refers to the highest dose strength, a physiological pH range between pH 1 to 8, an experimental temperature of 37°C, and the volume of 250 mL to be used. Apart from this basic information on the requirements to demonstrate high solubility, no further recommendations are given, for example, with respect to experimental methods and/or documentation.

In addition, it is particularly mentioned that polymorphism and particle size are to be considered although no further details are given. The recommendation may be related to situations where the active substance used in test and reference products differs regarding polymorphs and/or particle size.

Permeability

It is interesting to note that the European guidance particularly requires linear and complete absorption rather than demonstration of high permeability. Furthermore, the procedure for meeting this requirement remains the applicant's responsibility since no experimental settings are either recommended or discouraged. The importance of the linearity of absorption may be questioned since product-related documentation of BCS-based biowaiver (i.e., comparative in vitro dissolution) must be generated for every strength of a product series. This is in contrast to proportionality-based biowaiver, in which proving in vivo bioequivalence for just one dosage strength can be applied to other dosage strengths under certain provisions. Particular requirements for proportionality-based biowaiver are outlined in section 5.4 of the NfG.

In Vitro Dissolution

Comparative in vitro dissolution of the products in question is briefly addressed. Accordingly, similarity of dissolution is assumed (without additional statistics) if at least 85% of the labeled amount has been released for the test and reference product. However, no upper time limit is provided to define the benchmark for rapid dissolution. For example, if in vitro dissolution requires 45 minutes to result in 85% dissolution, this would still meet basic pharmacopoeial dissolution criteria for immediate-release dosage forms [see European Pharmacopoeia, Ph Eur (6)] and be acceptable under the EMEA NfG, but would exceed the 30 minutes as mentioned, for example, in the U.S. FDA guidance on BCS.

Formulation Considerations

Requirements on excipients are addressed generally in a short paragraph, that is, "well established compounds should be used in usual amounts and no interactions with the pharmacokinetics of the active drug substance should be expected." Required information on the manufacturing process includes

specifically the need to address possible effects on bioavailability of the drug substance in question.

Current Status

The first BCS-based biowaiver for a generic drug product, for example, in Germany was granted in 2002 and published (7). However, the limitations of the guideline have been recognized by regulators and industry, along with a rising interest in the possibility of filing BCS-based biowaiver for generic drug applications. Accordingly, a concept paper was released in 2007 (8), addressing currently missing aspects and supporting a revision of guideline recommendations specifically with respect to waiving bioequivalence studies, on the basis of the BCS concept. In particular, it was requested that the following issues be addressed in a revised document.

Drug Substance Considerations

- Which characteristics are deemed indispensable to prove a drug substance eligible for the BCS-based biowaiver approach and what kind of data (literature and/or experimental) are acceptable, for instance (and in addition to established guideline requirements):
 - Discuss “risk of bioinequivalence”
 - Define dose to be investigated in terms of solubility
 - Discuss whether BCS-based biowaiver may be acceptable within a restricted dose range due to solubility limitations, that is, biowaiver for lower strengths and in vivo bioequivalence study for higher dose strengths
 - Define permeability and/or absorption requirements
 - Discuss/clarify acceptance or exclusion of biowaiver extensions, for example, BCS-based biowaiver for BCS class II and/or III drugs

Drug Product Considerations

- Comprehensive description of in vitro dissolution requirements
 - Experimental setting, method validation
 - Evaluation of absence of product differences (or product “similarity”)
 - Delineation from in vitro/in vivo correlations and quality control
- Specification of the number of batches to be investigated
- Specification of how excipients are to be evaluated
- Clarification regarding fixed-dose combinations and prodrugs
- Clarification on the applicability of the BCS-based biowaiver approach (generic applications, drug development, variations)

This concept paper revealed the necessity for more detailed guidance on how a BCS-based biowaiver can be successfully achieved in European countries. As of this writing, it has been used as the basis for a new European guidance document on biowaiving based on the BCS, which is drafted as a separate appendix to the revised bioequivalence guideline (9).

THE WHO GUIDANCE

The BCS concept received pronounced attention by WHO experts drafting revised bioequivalence guidance documents during the last couple of years. Actually, the BCS-based biowaiver approach has been recognized here as a

TABLE 2 Eligibility of the BCS-Based Biowaiver According to the Current WHO Guidance Documents

	D:S 250 mL	
	BCS class I	BCS class II
	Highly permeable	Highly permeable
	Highly soluble	Poorly soluble
	Eligible	Eligible only if the D:S is 250 mL or lower at pH 6.8
85% abs		
	BCS class III	BCS class IV
	Poorly permeable	Poorly permeable
	Highly soluble	Poorly soluble
	Eligible if very rapidly dissolving	Not eligible

Abbreviations: WHO, World Health Organization; BCS, Biopharmaceutics Classification System.

useful tool to improve the quality of multisource (generic) pharmaceutical products and to ensure their interchangeability [see WHO Technical Report Series No. 937 Annex 7 (10) and 8 (11)]. The currently available documents outline the requirements, which are basically structured according to those of the U.S. FDA. However, recent findings as well as discussions on the possibility of biowaiver extensions (12–14) are implemented and specific requirements have been included accordingly.

While the BCS-based biowaiver approach has been possible only for highly soluble and highly permeable (BCS class I) drug substances, the WHO guidance documents (10,11) open the concept for BCS class III (highly soluble and limited permeability) drug substances and certain BCS class II (limited solubility and high permeability) drug substances, as shown in Table 2.

Like the U.S. FDA and the European guidance, section 5.5 of Annex 7 outlines the necessity of a risk assessment to minimize incorrect biowaiver decisions; however, this section is more comprehensive than the two previously discussed guidance documents. The WHO risk assessment includes consideration of, for example, therapeutic indications, known pharmacokinetic variations, and food effects. In addition, section 5.1 of Annex 8 mentions

- “critical use” medicines,
- narrow therapeutic index drugs,
- evidence of bioavailability problems or bioinequivalence related to the API, and
- polymorphism or excipients or pharmaceutical processes in manufacturing

as possible reasons to perform in vivo bioequivalence testing (apart from formulation-related issues).

Solubility

Like in the aforementioned two guidance documents, the definition of high solubility refers to the highest dose strength available on the market. However, reference is made to the highest dose recommended by WHO if the drug substance appears on the *WHO Model List of Essential Medicines*. Accordingly, the highest dose strength to be investigated may differ in this list from those used in certain local markets.

The pH range of 1.2 to 6.8 is reduced as compared to the U.S. FDA and European guidances but is considered sufficient and appropriate based on latest scientific findings and discussions. Solubility experiments should consider a maximum volume of 250 mL and a temperature of 37°C, which is in line with initial requirements.

Permeability

Recommendations focusing on permeability basically refer to the U.S. FDA guidance although possibilities are less extensive. Accordingly, absorption may be demonstrated by means of

- in vivo intestinal perfusion in humans, or
- in vitro permeation using excised human or animal intestinal tissue.

Supportive data may be generated by means of

- in vivo or in situ intestinal perfusion using animal models, or
- in vitro permeation across a monolayer of cultured epithelial cells (e.g., Caco-2) using a method validated using drug substances with known permeabilities.

It is outlined that scientific findings justify relaxation of the initially required value of 90% to 85% absorption, which may slightly extend the number of eligible compounds. For example, paracetamol, acetylsalicylic acid, lamivudine, and promethazine are now included in BCS class I instead of their initial U.S. FDA classification as BCS class III drug substances.

In Vitro Dissolution

Generally reference is made to *The International Pharmacopoeia* (15). However, it is specifically mentioned that dissolution tests recommended for quality control may not be suitable for comparison of multisource and comparator products in terms of bioequivalence.

Drug product dissolution is categorized as being “very rapid” or “rapid.” Generally, very rapid (at least 85% within 15 minutes) or rapid (at least 85% within 30 minutes) in vitro dissolution is required at every condition, that is, at pH 1.2, 4.5, and 6.8 to justify a BCS-based biowaiver of a multisource product and the respective comparator. Specific dissolution requirements are outlined depending on the properties of the drug substance since the BCS-based biowaiver approach has been extended beyond BCS class I drugs.

Accordingly, rapid or very rapid in vitro dissolution is acceptable for dosage forms of BCS class I drug substances. In contrast, very rapid dissolution is required for dosage forms with highly soluble drug substances exhibiting limited absorption (BCS class III).

Moreover, dosage forms with drug substances that are highly soluble at pH 6.8 but not at other pH and that are highly permeable (essentially BCS class II compounds with weak acidic properties) may be eligible for a BCS-based biowaiver approach if they are rapidly dissolving at pH 6.8. In addition, similarity of in vitro dissolution profiles of the multisource product and comparator should be demonstrated at pH 1.2 and 4.5, although rather low dissolution is expected due to solubility characteristics of respective drug substances, thereby preventing sink conditions, particularly at acidic pH. However, possible formulation differences

may become obvious under acidic experimental conditions like, for example, the impact of certain surfactants used in the formulation(s).

Formulation Considerations

Corresponding to the previously mentioned guidances, excipients are to be critically evaluated in terms of type and amounts. However, this evaluation is specifically explained to be a required risk assessment, which, together with the requirements stated above, should ensure an acceptable benefit-risk balance when in vivo bioequivalence testing is waived on this basis.

Current Status

Currently the WHO guidance on the BCS-based biowaiver is certainly the most up-to-date document considering recent scientific discussions and findings. Efforts have been made to implement this concept in the regulatory framework of developing countries to facilitate respective applications and thereby product quality.

Meanwhile, the WHO Prequalification Project, which focuses especially on medicines for the treatment of malaria, HIV/AIDS, tuberculosis, and for reproductive health, has recognized the need for specific drug substance-related guidance with respect to BCS-based biowaiver applications. These recommendations aim to account for the need to facilitate the appropriate use of the BCS-based biowaiver and at the same time to account for any special properties of drug substances and formulations to minimize the risk of false bioequivalence decisions. Currently, respective guidance documents have been released expressing the eligibility for BCS-based biowaiver applications for immediate-release formulations containing the following APIs as single components (16):

- Antiretroviral medicines
Lamivudine (BCS class I)
Stavudine (BCS class I)
Zidovudine (BCS class I)
- Antituberculosis medicines
Ethambutol (BCS class III)
Isoniazid (BCS class III/I)
Levofloxacin (BCS class I)
Ofloxacin (BCS class I)
Pyrazinamide (BCS class III/I)

OTHER JURISDICTIONS

Some countries are considering the BCS-based biowaiver concept by adopting either the U.S. FDA or European requirements on bioequivalence, that is, including at least basic information on BCS-based biowaiver applications.

Accordingly, *Australia* and *ASEAN* (the Association of Southeast Asian Nations) *countries* adopted the European guideline, thereby allowing application of the BCS-based biowaiver for BCS class I drug substances in immediate-release dosage forms.

Regarding *South Africa*, the BCS-based biowaiver approach is mentioned in a guidance on biostudies effective since June 2007 (17). The BCS-biowaiver approach is also implemented in a guideline on “dissolution” that includes the

BCS concept among other biowaiver options (18). This guidance document is expected to come into operation during 2008. Both documents basically refer to the U.S. FDA guidance on the BCS-based biowaiver.

India drafted a document “Guidelines for Bioavailability & Bioequivalence Studies” (March 2005) (19) where the basic requirements on solubility, absorption, and in vitro dissolution are rather briefly mentioned as an option to prove bioequivalence. Basic requirements are in line with the current U.S. FDA guidance on BCS-based biowaiver.

The *Pan American Health Organization* Working Group on Bioequivalence, drafted a document “Science based criteria for bioequivalence in vivo and in vitro, Bio-waivers, and strategic framework for implementation” basically adopting U.S. FDA recommendations as far as the BCS-based biowaiver is concerned (20).

Saudi Arabia drafted a guideline in 2005, also implementing the basic possibility to apply the BCS-based biowaiver for BCS class I drug substances manufactured in immediate-release formulations (“Bioequivalence Requirements Guidelines” Draft 2005) (21).

Other jurisdictions do not accept the BCS-based biowaiver approach or any of the current guidelines that have implemented it. Accordingly, *Switzerland*, *Canada*, and *Japan* have not implemented *bis dato* the BCS-based biowaiver as a means to ensure bioequivalence of different drug products in any shape or form.

DISCUSSION

The basic principles that are to be addressed when filing a BCS-based biowaiver are widely appreciated, but still there are not as many BCS-based applications as was perhaps initially expected. Ongoing scientific discussions on the BCS concept including subclassifications and classifications based on metabolic properties (14,22) may contribute to the underutilization of BCS-based biowaivers (23). Another reason for limited implementation to date stems from the slightly divergent requirements in various jurisdictions—as evident from the comparison of the various guidances (Table 3). A reason that the detailed requirements are not uniform is the diversity of opinion about which data are scientifically sufficient and justified as a surrogate for in vivo bioequivalence. With respect to the drug substance, the question of which properties are conducive to demonstration of product similarity using dissolution tests is still being discussed. And with respect to dissolution test requirements, part of the diversity in opinion arises from their twofold application: on the one hand to test pharmaceutical quality and on the other hand to assess limits to in vivo performance. As a result, in vitro dissolution experiments required in the framework of BCS-based biowaiver may not necessarily meet the same criteria applied to test pharmaceutical quality.

In vitro dissolution experiments generated as part of the BCS-based biowaiver application cannot be interpreted as a kind of in vitro/in vivo correlation—far more, they represent compliance or noncompliance as determined by a cutoff value. Accordingly, dissolution profile differences should not be discussed in terms of their in vivo relevance. Therefore, the ultimate goal of the BCS-based biowaiver should be emphasized, that is, demonstrating bioequivalence by justifying the absence of differences between two formulations.

TABLE 3 Comparison of the Requirements of the U.S. FDA, the EMEA, and the WHO

	U.S. FDA	Europe EMEA	WHO
Risk assessment required	x	x	x
Eligible drug substances	BCS class I	BCS class I	BCS class I, III and II if highly soluble at pH 6.8 and highly permeable
Definition of "high permeability"	90% Absorption	Linear and complete absorption	85% Absorption
pH range to be considered for proving "high solubility"	pH 1–7.5	pH 1–8	pH 1–6.8
In vitro dissolution			
Experimental conditions	Ref. to USP	No recommendations	Ref. to International Pharmacopoeia
pH range to be considered	0.1 N HCl pH 4.5 buffer pH 6.8 buffer	pH 1.0 pH 4.5 pH 6.8	pH 1.2 HCl solution pH 4.5 acetate buffer pH 6.8 phosphate buffer
Volume of dissolution medium	900 mL	—	900 mL
Agitation conditions	Paddle app.: 50 rpm Basket app.: 100 rpm	—	Paddle app.: 75 rpm Basket app.: 100 rpm
Data evaluation	Profile comparison using f_2 testing except in case of very rapid dissolution (85% within 15 min or less)	Profile comparison using f_2 testing or other except in case of very rapid dissolution (85% within 15 min or less)	Profile comparison using f_2 testing or other except in case of very rapid dissolution (85% within 15 min or less)

Abbreviations: FDA, Food and Drug Administration; EMEA, the European Medicines Evaluation Agency; WHO, World Health Organization; USP, United States Pharmacopoeia; app., apparatus.

In the last few years, the Federation Internationale Pharmaceutique (FIP) BCS working group has published a number of so-called biowaiver monographs (24–37) to facilitate successful filing of respective submissions for marketing authorization of immediate-release dosage forms. Comprehensive literature surveys have allowed BCS classification of several drug substances. In addition, peculiarities related to the drug itself and/or related to formulation that may be relevant in terms of bioavailability and bioequivalence are explained.

Apart from controversial views on eligibility of BCS classes and required experimental investigations regarding solubility, absorption, and in vitro dissolution, there are still questions that remain unsolved or insufficiently addressed in the time being. These questions relate to drug substances as well as formulation characteristics.

For example, recommendations on how to handle prodrugs are clearly addressed only in the U.S. FDA guidance (1). Here it is outlined that the permeability classification should be done according to the site of conversion to the drug. Hence, permeability of the prodrug is relevant in cases where conversion occurs after its transport through the epithelium, and permeability of the active

drug is relevant in cases where conversion takes place prior to uptake across the gut mucosa.

Another frequent though open question is how the dosage strength should be considered in terms of solubility classification. For example, is it reasonable to apply the BCS-based biowaiver for a lower strength but to conduct in vivo bioequivalence studies for higher strengths if the API solubility at the higher strength does not comply with the cutoff for solubility?

Moreover, handling of fixed-dose combinations is inadequately addressed in this framework. According to WHO regulations [Annex 7 (10) (section 5.1 (d))], a BCS-based biowaiver is applicable only in those cases where all drug substances in a combinational product are classified as eligible, that is, an in vivo bioequivalence study is necessary if the classification of one compound requires it. Other BCS guidance documents do not address this item sufficiently, if at all.

Finally, the European Directive 2001/82 as amended (38) requires the same qualitative and quantitative composition in active substances for a “generic medicinal product” as compared to the reference product. However, it is defined that “different salts, esters, ethers, isomers, mixtures of isomers, complexes or derivatives of an active substance shall be considered the same active substance unless they differ significantly in properties with regard to safety and/or efficacy.” It may be questioned—but is not addressed in the current guidance—whether this definition of a generic active drug substance may be fully applicable in the BCS-based biowaiver concept too. It is deemed strongly advisable to restrict the eligibility of a BCS-based biowaiver to the identical active drug substances, in particular when considering possible biowaiver extension to BCS class II and III compounds.

SUMMARY AND FUTURE PERSPECTIVES

The BCS-based biowaiver concept has been discussed in the scientific community for more than 10 years and has been implemented in several guidance documents worldwide. However, appropriate use of this possibility to, for example, apply for approval of generic drugs is still limited due to several reasons, one of which is the lack of acceptance *bis dato* in a few countries. It may be hoped that harmonization processes would include the BCS-based biowaiver as a means of proving bioequivalence where necessary, thereby reducing the number of unnecessarily performed in vivo bioequivalence studies. It is anticipated that revised and/or new regulatory guidance documents would implement current scientific findings thus facilitating well-founded biowaiver applications. The intention of the U.S. FDA to revise its first regulatory guidance on BCS, as expressed at the American Association of Pharmaceutical Scientists (AAPS)/FDA meeting on bioequivalence and BCS in May 2007 (39), as well as the current revisions in the European Union, are promising developments in this regard. Keeping in mind that the general principles concerning application of the biowaiver-based approval are addressed in all regulatory guidelines, the WHO approach seems to be currently the most promising, in that it advises how to weigh the potential risks and benefits of applying the biowaiver concept in a drug substance-specific manner.

Disclaimer: This text includes personal opinions of the author, which do not necessarily represent the views or policies of the German Federal Institute for Drugs and Medical Devices (BfArM).

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Biowaiving Based on In Vitro-In Vivo Correlation

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INTRODUCTION

Biowaiving—that is, approval of a drug product without having to conduct an in vivo bioequivalence (BE) study—is a well-recognized process of reducing regulatory burden. However, it should not be done at the cost of product quality. To date, there are applications of biowaiving that can be utilized to provide regulatory relief without loss of drug product quality. These include:

- Biowaiver for certain class of drugs based on biopharmaceutics classification system (BCS) (1);
- Biowaiver for lower strengths of immediate release (IR) and extended release (ER) dosage forms based on formulation proportionality and similar dissolution profile (2); and
- Biowaiver based on in vitro-in vivo correlation (IVIVC).

In this chapter, the primary focus will be the last aspect, biowaiving based on IVIVC.

BCS-BASED BIOWAIVER

The FDA guidance for Industry “Waiver of in vivo bioavailability and bioequivalence studies for IR solid oral dosage forms based on Biopharmaceutics Classification System (BCS), August 2000” is the only guidance that includes the words “waiver of in vivo studies” in the title of the guidance, signifying the importance of BCS (1).

The BCS classified the drug substances (API) into four binary classes, on the basis of combinations of high or low drug solubility and drug permeability characteristics:

Class 1: High solubility/high permeability (HS/HP)

Class 2: Low solubility/high permeability (LS/HP)

Class 3: High solubility/low permeability (HS/LP) and

Class 4: Low solubility/low permeability (LS/LP)

One of the most important applications of BCS is biowaiver of multisource (generic) drug products. The FDA guidance proposes biowaiver only for drug products containing BCS class 1 APIs. This is viewed by many as very conservative. Using sound scientific judgment, and after extensive discussions and deliberations with experts in the area, the World Health Organization (WHO) developed guidelines “Multi-source (generic) pharmaceutical products: Guidelines on registration requirements to establish interchangeability” (3). The aim was to globally reduce regulatory burden without sacrificing the product quality.

The WHO guideline proposes that the dissolution test for multisource (test) and comparator (reference) product be carried out using paddle method at

75 rpm or basket method at 100 rpm, in 900 mL (or less) of dissolution medium, at pH 1.2, 4.5, and 6.8. In the WHO guideline, a paddle speed of 75 rpm is recommended to avoid coning effects and unnecessary variability in dissolution test results. For biowaivers, test and reference products must have similar dissolution profiles in all three media—pH 1.2, 4.5, and 6.8 or be “very rapidly dissolving.”

For use and application of BCS, the dissolution is characterized into three categories:

- Very rapidly dissolving—85% dissolution in 15 minutes
- Rapidly dissolving—85% dissolution in 30 minutes
- Not rapidly dissolving—more than 30 minutes needed for 85% dissolution

The WHO approach further ensures the quality of the drug product, by stipulating that it be manufactured under GMP conditions. It is proposed that biowaiver can be granted for the following multisource drug products under the following conditions:

- BCS class 1 (HS/HP): Drug products with very rapid dissolution or rapid dissolution in pH 1.2, 4.5, and 6.8
- BCS class 2 (LS/HP/weak acids): Drug products with rapid dissolution in pH 6.8 and similar dissolution profiles of test and comparator product at pH 1.2, 4.5, and 6.8
- BCS class 3 (HS/LP): Drug products with very rapid dissolution in pH 1.2, 4.5, and 6.8. In addition, this class of drugs should not contain any excipients that are known to alter GI motility and/or absorption

For biowaivers, the multisource (test) and the comparator (reference) products must have similar dissolution profile (f_2) in all three media. The similarity factor f_2 is calculated using the following equation:

$$f_2 = 50 \text{LOG} \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^n (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

where R_t and T_t are the cumulative percentage dissolved at each of the selected n time points of the reference and test product, respectively. The f_2 is inversely proportional to the average squared difference between two dissolution profiles and measures the closeness between the two profiles. When the two profiles are identical, $f_2 = 100$. An average difference of 10% at all measured time points results in an f_2 value of 50. FDA has set a public standard of f_2 value to be between 50 and 100 to indicate similarity between two dissolution profiles (4).

In addition, the WHO guideline requires the sponsor to submit a risk analysis that includes consideration of the risks of misjudging a bioinequivalent drug product as bioequivalent and the therapeutic consequences of substituting a bioinequivalent drug product.

Biowaivers based on the BCS system are applicable only to IR, nonnarrow therapeutic index drug products. By contrast, the BCS-based biowaiver is not applicable for modified release (ER) drug products. Instead, in vivo BE studies for products with modified release products can be waived on the basis of an

IVIVC. There is a fundamental basic difference between the two “biowaiver” procedures. The BCS-based biowaiver requires *in vitro* dissolution profile comparison of the multisource (test) product with the reference (innovator) product according to a set of standard dissolution test conditions prescribed by the regulatory authority, but does not require any additional *in vivo* studies. On the other hand, an IVIVC-based biowaiver is product and formulation specific (for a given product and manufacturer), requires the sponsor to perform additional *in vivo* studies to establish a correlation between *in vitro* and *in vivo* studies, and may be based on dissolution test conditions chosen by the manufacturer.

Dissolution as the rate-limiting step for drug absorption is essential for establishing an IVIVC. For BCS classes 1 and 3 (highly soluble) drug products housed in IR formulations, dissolution is not the rate controlling step to absorption, and therefore IVIVC is not feasible for BCS classes 1 and 3 drugs in such products. However, when BCS classes 1 and 3 drugs are formulated as ER products, an IVIVC can often be achieved. For IR products containing BCS class 2 (LS/HP) drugs, dissolution is the rate-limiting step for drug absorption, and therefore it is often possible to obtain IVIVC for these products. In the case of IR products containing BCS class 4 drugs, dissolution rate may partly limit absorption but permeability is also likely to be a limiting factor to absorption, so application of IVIVC to obtain an approval without further *in vivo* testing is unlikely to be as easy to achieve as with MR formulations or IR products containing BCS class 2 drugs.

IN VITRO-IN VIVO CORRELATION

IVIVC is a functional or qualitative relationship between *in vitro* release and *in vivo* bioavailability parameters. The goal of developing an IVIVC is to establish a set of *in vitro* dissolution/release specifications that guarantee the *in vivo* performance of a product (predictability) and thus obviate the need to perform further *in vivo* BE studies to obtain/maintain regulatory approval.

Development of IVIVC is possible when the *in vitro* drug release is the rate-determining step for the subsequent drug absorption. One or more *in vivo* parameters are correlated with *in vitro* release parameter(s) of the product. To achieve such a correlation, it is necessary to test products that differ in *in vivo* as well as *in vitro* performance. Differences in the *in vitro* release profile should be reflected in differences in the *in vivo* performance of the product. An optimum correlation is one in which *in vitro* dissolution is predictive of *in vivo* behavior of various lots of products in the target population. In general, for an IVIVC to be established, a minimum of three batches (products) differing in *in vivo* and *in vitro* performance are needed. If only two batches (products) are considered, one refers to a “rank order relationship” or an “association” rather than a correlation.

IVIVC serves as a developmental tool for the formulator. It provides better understanding of critical formulation and process elements, helps to reduce the extent of dissolution work, provides a basis for setting biorelevant *in vitro* release specification, and reduces the investment required for line extensions (additional strengths) and later product changes. IVIVC is a credible tool to select discriminating *in vitro* test conditions and to set therapeutically meaningful *in vitro* release specifications. Applied correctly, the IVIVC can save

substantial time and costs both during the development of a new IR or ER product and when registering product changes, since, once the IVIVC is established, further in vivo BE studies will not be needed.

In vitro parameters can include dissolution/release profile (% released vs. time), the amount of drug dissolved at a specified time-point, time required for a specified % release or the dissolution rate itself. The in vivo parameters can include $C_{\max}$, $t_{\max}$, AUC, Ae, and/or % absorbed vs. time, depending on the correlation level (see later). For level A and B correlations, the type of dissolution data to be generated is fixed, but the experimental conditions to obtain the data may be varied. For level C correlations, the in vitro parameters can be selected by the sponsor, and here, too, the parameter values will change according to the in vitro methodology selected (apparatus, composition, and volume of medium, etc.). Selection of in vivo parameters is more restricted and dependent on the correlation level applied. Knowledge of GI physiology, the rate-limiting step to drug absorption, and the site(s) of absorption of the drug in the gastrointestinal tract will all be useful in designing experiments to establish an IVIVC.

There are three levels of IVIVC (level A, B, and C) described in the United States Pharmacopeia (5) and four levels of IVIVC (level A, B, C, and multiple level C, often referred as level D) described in FDA guidance (6). These were developed primarily with ER product evaluation in mind.

- Level A: Functional relationship between in vitro dissolution and the in vivo input rate, correlation of profiles, linear or nonlinear relationship. A level A correlation is usually estimated by a two-stage procedure—deconvolution followed by comparison of the fraction of drug absorbed to the fraction of the drug dissolved. It is generally linear and is a point-to-point relationship between in vitro dissolution and in vivo input rate. This is the most useful type of correlation from both a regulatory and scientific point of view, since it is the most data-rich of the different levels.
- Level B: Correlation based on statistical moment analysis (i.e., mean in vitro dissolution time is correlated with mean in vivo residence time). Level B correlations are least useful for regulatory purposes.
- Level C: Single-point relationship between a dissolution parameter (e.g., T 50% dissolved in 1 hour) and one pharmacokinetic parameter (e.g., AUC, $C_{\max}$)
- Level D: Multiple level C correlation relates to one or several pharmacokinetic parameters of interest to the amount of drug dissolved at several time points of the dissolution profile. This type of correlation often is referred as level D correlation.

The FDA guidance on IVIVC discusses the development of an IVIVC and evaluation of its ability to predict in vivo performance, provides guidance for setting dissolution specifications based on IVIVC, describes application of IVIVC as a surrogate for in vivo BE, and indicates when it is necessary to document BE during the initial approval process or because of certain pre- or postapproval changes, for example, formulation, equipment, process, and manufacturing site changes. The guidance also discusses ways of determining and evaluating internal and external predictability.

The most important application of level A correlation is biowaivers. The FDA guidance indicates that the most common process for developing a level A

IVIVC is to (i) develop formulations with different release rates, such as slow, medium, and fast (or a single release rate if dissolution is condition independent); (ii) obtain *in vitro* dissolution profiles and *in vivo* plasma concentration profiles for these formulations; and (iii) estimate the time-course of *in vivo* absorption or dissolution time using an appropriate deconvolution technique for each formulation and subject (e.g., Wagner-Nelson, numerical deconvolution). These three steps establish the IVIVC model (6).

An IVIVC should be evaluated to determine how well the *in vivo* performance of a drug product can be predicted from its *in vitro* dissolution characteristics, and whether this level of predictability is maintained over a range of *in vitro* dissolution release rates and manufacturing changes (within the design space). This approach focuses on the estimation of predictive performance or, conversely, prediction error. Evaluation of internal predictability is based on the initial data used to define the IVIVC model. Evaluation of external predictability is based on additional test data sets. Application of one or more of these procedures to the IVIVC modeling process constitutes evaluation of predictability.

A single-point level C correlation can be used for setting dissolution specification, but not for biowaiver. A multiple-point level C correlation may be used to justify a biowaiver, provided that the correlation has been established over the entire dissolution profile with one or more pharmacokinetic parameters of interest.

Although it is not an FDA requirement to develop an IVIVC for ER dosage forms, sponsors are encouraged to develop one. Drug product approval does not depend on having an IVIVC, but it will most likely be expected for characterizing the quality of the product formulation, the relevance of the dissolution testing conditions and for setting dissolution specifications in assuring product similarity after scale-up and post-approval changes (SUPAC) (7). Without an IVIVC, the sponsor would be required to carry out *in vivo* studies on the changed formulation. Note that since these correlations are product specific and formulation specific, they cannot be applied when there is a fundamental change in the release mechanism.

It is not easy to develop a level A correlation, as it is a time consuming and expensive process. Therefore sponsors often opt to carry out the *in vivo* studies (after SUPAC related changes), instead. However, once an IVIVC is developed, it can encompass the many minor changes that will be necessary to the product in the course of its life cycle and the initial investment in establishing the IVIVC will pay off handsomely.

As mentioned above, development of IVIVC is possible when the *in vitro* drug release is the rate limiting step for the subsequent drug absorption. Traditionally, ER dosage forms aim to control the absorption rate of the drug by controlling the rate of release from the dosage form, so these are generally good candidates for an IVIVC. Most of the work on IVIVC that has appeared to date in the literature and which has been used to regulatory advantage has focused on ER formulations. Recently, much interest has been expressed in the application of IVIVC to IR products containing BCS class 2 drugs, since in this case too, the rate controlling step to absorption often lies in the release/dissolution from the dosage form. In some cases of BCS class 4 APIs, where the permeability of the API is moderate to good, although unable to fulfill the requirements for "highly permeable", it may also be possible to achieve an IVIVC for an IR product (1). Indeed, IVIVCs of the level C type have been achieved for several IR products

made by different manufacturers, including digoxin, prednisone, tetracycline, phenytoin and carbamazepine. These correlations have had practical as well as regulatory implications; establishing an IVIVC resulted in establishing in vitro dissolution specifications that are relevant to in vivo performance, and thus in improved product quality.

Attempts have also been made to develop IVIVC for non-oral dosage forms such as Topical and Transdermal dosage forms, but have met with limited success. In many instances, only two products were utilized to generate correlations for topical dosage forms,, and therefore only a rank order association could be achieved.

CONCEPT OF MAPPING: EXPLORING THE RESPONSE SURFACE

During the manufacturing process, formulators use drug and excipient specifications together with critical manufacturing and process variables to develop a robust formulation and develop a 'response surface' which assures the formulator to be in a 'safe zone' during manufacturing process (8). This safe zone, constructed using various combinations of manufacturing and process parameters, assures product performance. Formulations within this safe zone may exhibit different in vitro dissolution profiles. Products with extreme in vitro dissolution profiles are then studied with respect to their in vivo performance to establish an acceptable response surface and IVIVC. In other words, the dissolution performance is 'mapped' onto the in vivo performance. The mapping approach thus provides the information on the boundary lines between acceptable and unacceptable dissolution characteristics and the boundaries are used to set dissolution specifications. This mapping concept can be applied to both ER and IR dosage forms, depending on BCS characteristics of the drug.

QUALITY BY DESIGN AND THE DESIGN SPACE

Pharmaceutical Quality by Design (QbD) is a systematic, scientific, risk-based, holistic and proactive approach to pharmaceutical development that begins with predefined biopharmaceutic objectives and with emphasis on product and process understanding and control (9). QbD identifies general characteristics that are critical to quality from the perspective of the patient, translates them into the attributes that the drug product should possess, and establishes to what extent the critical process parameters can be varied without compromising the ability to consistently produce a drug product with the desired characteristics. In order to achieve this, the relationship between formulation and manufacturing process variables and product characteristics need to be established and sources of variability need to be identified. Generally, the product and process characteristics are derived from a combination of prior knowledge and experimental assessment during product development (9,10). This knowledge is then used to implement a flexible and robust manufacturing process that can be adapted if necessary to ensure product consistency over time.

Process parameters refer to input operating parameters, for example, for tableting operations these might include aspects such as feed rate and compression pressure as well as tooling specifications. As it is often the case that there are interactions among several critical process parameters, establishing a design space is a useful approach to ensure product quality. The current

definition of a design space is “the multidimensional combination and interaction of input variables and process parameters that have been demonstrated to provide assurance of quality”. A design space is thus a way to represent the process understanding that has been established. When a design space is submitted to the FDA, it can result in approval to operate at all points within that design space without any need to reapply for continued regulatory approval. Specifically, working within the approved design space is not considered as a “change” from a regulatory point of view. By contrast, movement out of the design space is considered to be a “change” and would normally initiate a regulatory post approval change process. In other words, products manufactured within “Design Space” can be considered as fulfilling the criteria for a biowaiver (continued product approval without the necessity for a human BE study). The design space is proposed by the applicant and is subject to regulatory assessment and approval.

Design space – the established range of process parameters that has been demonstrated to provide assurance of quality from both a pharmaceutical technological and a biopharmaceutical perspective. Following the principles of QbD, one ends up in a safe area of manufacturing parameters that is then referred as the “Design space”. This provides the manufacturer to move freely within the space (gives an assurance of product performance), without any fear of having to resubmit the applications as a SUPAC related change. The benefits of having a design space are clear; the greatest challenge is the cost of establishing it.

IDENTIFYING THE DESIGN SPACE: ROLE OF IVIVC

The mapping concept provides a way of establishing the design space in a way that is relevant to in vivo objectives, using IVIVC as an important linking tool. Together, they aim at providing the flexibility to manufacture the product within the window of defined operating parameters to assure product quality relevant to in vivo performance. It would not be practically feasible to study the impact of changes in each and every operating parameter that could affect the design space in vivo study. By establishing an IVIVC, however, the impact of variations can be thenceforth assessed on an in vitro basis, resulting in huge savings in resources. The IVIVC can certainly help to “bridge” the biopharmaceutical requirements for the design space to the selection of formulation and process parameters.

QUALITY BY DESIGN IN ICH GUIDANCES

QbD is an essential part of the modern approach to pharmaceutical quality. The critical process parameters and operating parameters should be combined with critical material attributes to describe the relation between unit operation inputs and outputs. The International Conference on Harmonization (ICH) has developed three guidelines—Q8 Pharmaceutical development (9); Q9 Quality risk management (11); and Q10 Pharmaceutical quality systems (12). All are inter-related and deal with the development of a pharmaceutical product.

Q8: Product Development

This guideline focuses on the performance of the drug product and includes attributes such as identity, strength and purity (9). Q8 focuses on pharmaceutical development, product performance and QbD and gives guidance on how to set

specifications. Specifications are critical quality standards that are proposed and justified by the manufacturer and approved by regulatory authorities. The physicochemical and biological properties relevant to the safety, performance or manufacturability of the drug product should be taken into consideration. This includes the physiological drug implications of drug substance and formulation attributes.

Critical formulation attributes and process parameters are generally identified through an assessment of the extent to which their variation can have impact on the quality of the drug product. This scientific understanding can facilitate establishment of an expanded design space and provides opportunities to develop flexible regulatory approaches.

Q9: Quality Risk Management

This guideline offers a systematic approach to quality risk management (11). It specifically provides guidance on the principles and some of the tools of quality risk management that can enable more effective and consistent risk based decisions regarding the quality of drug substances and drug products across the product lifecycle. It is not intended to create any expectations beyond the current regulatory requirements. The guideline provides principles and examples of tools for quality risk management that can be applied to different aspects of pharmaceutical quality. These can be applied at all stages of product life cycle, including product development, manufacturing, distribution, inspection and submission/review process. Two important principles of quality risk management are (i) the evaluation of the risk to quality should be based on scientific knowledge and link to the protection of the patient and (ii) the level of effort and documentation should be commensurate with the level of risk. The process of quality risk management includes risk identification, risk assessment, risk control and risk reduction. The risk assessment and management can adapt management tools and procedures (e.g., standard operating procedures) to maintain drug product quality. Quality risk management is a process that supports science-based and practical decisions when integrated into quality systems; it can facilitate informed decisions.

Q10: Pharmaceutical Quality System

This guidance describes the modern quality systems needed to establish and maintain a state of control that can ensure the realization of a quality drug product and facilitate continual improvement over the life cycle of a drug product (12). This guideline serves as a bridge between different regional regulations, and establishes the process for implementing Q8 and Q9 guidelines, resulting in global harmonization of quality systems.

CONCLUSIONS

IVIVC has traditionally been employed to provide freedom to make needed formulation/manufacturing changes of ER dosage forms without the need to demonstrate BE in in vivo studies. IVIVC is also useful for setting appropriate dissolution specifications to assure product performance in vivo as well as the pharmaceutical quality. In the context of Quality by Design, IVIVC offers a linking (mapping) mechanism between formulation and process parameters and

the *in vivo* performance of the product and thus facilitates identification of the Design space. As long as manufacturer conforms to this design space, there is no further need to seek regulatory approval for any adjustments. It is expected that going forward, IVIVC will be increasingly used in this context to establish design spaces for both IR and ER dosage forms.

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Appendix: Use of Excel in Biopharmaceutic Analysis, Simulation, and Modeling

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From its nature, prediction and assessment of drug absorption is a multi-disciplinary task, involving physical chemistry, pharmacokinetics, biopharmaceutics, and many other fields. Most applications are based on a mathematical background.

- *Polynomials* are used for interpolation and numerical integration.
- *Distribution functions* describe the time profile of release in vitro and response in vivo. *Moments* summarize the profiles in terms of extent, rate, and shape.
- *Linear differential equations* describe pharmacokinetic models.
- *Systems analysis* generalizes the elementary superposition principle by convolution/deconvolution operations.
- *Statistics* provide tools for nonlinear regression, general linear models (GLM), and in vivo/in vitro correlation (IVIVC) or relationship (IVIVR).
- *Matrix* language presents a short-hand notation for all these items, with which mathematical connections become easier for non-mathematicians to understand.

These mathematical aspects, although important for drug product development and assessment, are covered only marginally by the literature: While over-represented in a manifold of sophisticated journal papers, there is an obvious lack of reviews and textbooks on the subject. Regarding computer software, the situation is likewise unsatisfactory. Commercial pharmacokinetic packages such as *WinNonlin*[®], *Kinetika*[®], or *Gastro Plus*[®] are available only with high cost; many important aspects of IVIVC/IVIVR are not included or not made transparent.

Given this situation, it is worthwhile to consider that MS Office is available worldwide at low cost and provides all facilities for data collection (*Access*), analysis (*Excel*), reporting (*Word*), and presentation (*PowerPoint*). In particular, Excel provides all needed analysis tools, including statistical analysis. In addition, *VisualBasic* (VBA) as general programming language provides a link to other software.

The CD-ROM attached to this book attempts to fill the gap between theoretical descriptions and sophisticated usage in journal articles by providing a documentation of relevant topics, separated spatially in two computer files:

- DOCUMENT.DOC discusses the mathematical background, with emphasis on matrix language and handling by Excel.
- WORKBOOK.XLS presents an Excel workbook with about 20 relevant worksheets. These may be used by any interested scientist and may be adjusted to his/her personal needs.

The documentation, primarily based on previous publications of the author, is not exhaustive but reflects practical aspects made during many years of active work in the field. As it is still in a process of elaboration, comments and cooperation are highly welcome.

Index

- AAPS. *See* American Association of Pharmaceutical Scientists (AAPS)
- AAPS/FDA Workshops, 207–209
findings from, 207–209
- Abbreviated New Drug Applications (ANDA), 141, 209, 374
- ABE. *See* Average bioequivalence (ABE)
- ABEsc. *See* Scaled average bioequivalence (ABEsc)
- Absorption
colonic drug
 drug degradation and, 274–275
 permeability and, 272–274
 preclinical risk assessment of, 272–275
 solubility and, 274
drug
 in small intestine, 13
 in stomach, 7
 microvilli in, 7, 10
 of nutrients, 2, 3–4, 10
 variability in, 351
 villi in, 7, 10
Absorption modeling
 in drug discovery and development, applications of, 342–351
 in formulation development, 301
Absorption potential (AP)
 calculation, 299
Absorptive cells, in small intestine, 12
ACAT model. *See* Advanced CAT (ACAT) model
Accelerator mass spectrometry (AMS), 289
Acceptance criterion
 defined, 362
Acquired immunodeficiency syndrome (AIDS)
 enteropathy, 133–134
Active pharmaceutical ingredients (API), 141, 211
ADR. *See* Adverse drug reactions (ADR)
- Advanced CAT (ACAT) model, 339–341
Adverse drug reactions (ADR), 214, 218
- AIDS. *See* Acquired immunodeficiency syndrome (AIDS)
- American Association of Pharmaceutical Scientists (AAPS), 207, 209, 212
- Amidases/proteases, 29
Aminopeptidase, 113
Amphoteric compounds, 208
AMS. *See* Accelerator mass spectrometry (AMS)
- Amylase, 10
Amyloidosis, 134
ANDA. *See* Abbreviated New Drug Applications (ANDA)
- Animal model
 for absorption. *See* Dogs; Intestinal permeability; Rat
 IR formulation, 318
 challenges and limitations, 327–329
 common models, 320–321
 dog model, 325–327
 importance of characterization, 319
 oral clinical formulations, 321–325
 in preclinical formulation development, 300–301
AP. *See* Absorption potential (AP)
- API. *See* Active pharmaceutical ingredient (API)
- Apparatus, with impeller and sampler, 238–239
Apparent permeability (Papp), 24
 coefficient
 effective permeability coefficient *versus*, 174–175
- Appendix, 4
Aquaporins, 54
Artificial membranes (PAMPA), 26
Asacolin[®], 255
ASEAN. *See* Association of Southeast Asian Nations (ASEAN)
- Association of Southeast Asian Nations (ASEAN), 380

- AstraZeneca, 265, 274–275
- Atovaquone
 bioavailability of, 100
- AUC. *See* Concentration-time curve (AUC)
- Average bioequivalence (ABE), 357–358
- BA. *See* Bioavailability (BA)
- Bacteria
 in colon, 15, 251
 dietary residues and, 17
 gastric fluid and, 5
 in small intestine, 1
- Baguette-based meal, 44
- Basket method. *See* USP apparatus 1
- BCRP. *See* Breast cancer resistance (BCRP)
- BCS. *See* Biopharmaceutics Classification System (BCS)
- BCS-based biowaiver
 background, 372
 European guidance, 375
 current status, 377
 formulation considerations, 376–377
 in vitro dissolution, 376
 permeability, 376
 solubility, 376
- U.S. FDA guidance, 372–373, 386
 current status, 375
 formulation considerations, 375
 in vitro dissolution, 374–375
 permeability, 374
 solubility, 374
- WHO guidance, 377, 386–387
 current status, 380
 formulation considerations, 380
 in vitro dissolution, 379–380
 permeability, 379
 solubility, 378–379
- BCS classification
 of drugs, 139, 146, 149–150
 global drug market and, 149
- BCS class II acidic drugs, biowaiver criteria for, 143
- BCS guidance, 206, 207, 209
- BDDCS. *See* Biopharmaceutical Drug Disposition Classification System (BDDCS)
- BE. *See* Bioequivalence (BE)
- Beagle dog model
 IR formulation, 325–327
- BE assessment. *See* Bioequivalence (BE) assessment
- Behcet's syndrome, 134
- Bicarbonate, in intestinal fluid, 14
- Bidirectional transport, 175–176
 pH gradient and, 193
 use of inhibitors in, 176
- Bile, 114
 acids
 in colon fluid, 17
 drug absorption and, 14
 fasted state intestinal fluid, 14
 fed state intestinal fluid, 14
 function of, 93
 hepatic metabolites and, 2
 role, 10
- Bile salts (BS), 93
 in colon, 16
 drug dissolution in fasted state and, 226
 in FeSSIF, 234
 in FSGES, 228
 surface tension and, 226
- Bioavailability (BA), 32, 139. *See also*
 Bioequivalence (BE) assessment
 defined, 140, 356
 in fed state, 227
 intestinal, 24, 29
 intra gastric environment and, 228
 oral, 21–22
- BioDis[®], 246–247
- Bioequivalence (BE), 138, 140–145, 206, 210, 213, 218, 224
 AUC requirements for, 140, 141
 C_{max} and, 140, 141
- Bioequivalence (BE) assessment
 approaches for evaluation of
 current FDA considerations for, 364–365
 EMA approach, 365
 individual BE, 359–360
 multiple-dose studies, 359
 point estimate of mean T/R ratio, 359
 replicate designs, 359
 scaled BE limits with leveling-off
 properties, 363–364
 scaled procedures, 360–363
 widening of acceptance limits to prefixed
 constant values, 360
 average bioequivalence (ABE), 357–358
 background, 356–357
 highly variable (HV) drugs and drug
 products, 358–365
 metabolites in, 365–367
 EMA guideline, 367
 FDA guideline, 367
 problem in establishing, 358–359
 simulated BE studies, 365–367

- Bioequivalence (BE) studies
 in biowaiver, 140–145
 food-effect studies for, 147
 in vivo and in vitro, 145
- Bioequivalence (BE) testing, 212–220
 assessment of drug and, 216–217
 cost of, 213–215
 ethical considerations in, 218–219
 in vitro and in vivo studies in, 212–220
- Biopharmaceutical Drug Disposition Classification System (BDDCS), 96, 207
- Biopharmaceutical preformulation
 in vivo human study techniques, 275–276
 preclinical regional drug absorption assessment, 272–275
- Biopharmaceutics Classification System (BCS), 95–96, 138, 172, 206–220, 224, 270, 273, 296, 338
 BDDCS and, 212
 in BE regulation, 138, 141, 147
 biowaiving. *See* BCS-based biowaiver
 class 1 drugs, 96–97
 class 2 drugs, 97–99
 class 3 drugs, 99
 class 4 drugs, 99–100
 in drug design, 146
 in drug development, 145–149, 211–212
 drug quality and, 139, 149
 FIM formulation development flow charts based on, 305
 four classes of drugs, 386
 impact of
 AAPS/FDA workshops and, 207–209
 EMA draft guidelines and, 210–211
 FIP biowaiver monograph series, 210
 WHO activities and, 209–210
 overview, 297
 principles, in formulation development, 297–299
- Biorelevant dissolution media
 small intestine, drug dissolution in, 231–235
 stomach, drug dissolution in, 225–231
- Biorelevant dissolution system, 245
- Biorelevant solubility, 155
- Biowaivers, 140–145, 206, 207, 208, 209, 210, 214, 218
 BCS approach to, 141–14
 for BCS class I drugs, 142–143
 for BCS class II drugs, 143
 for BCS class III drugs, 143–145
 BE studies in, 140–141
 extensions, 206, 207
 guideline, 224
- [Biowaivers]
 monograph series, FIP, 209–210
 for pregabalin, 141
 for sotalol hydrochloride, 141–142
- Biowaiving. *See also* BCS-based biowaiver
 based on IVIVC, 388–391
 defined, 386
- Breast cancer resistance (BCRP), 115
 transporters, 27–28
- Brush border, 114–115
- BS. *See* Bile salts (BS)
- Budesonide, 275
- Buffer capacity
 of colonic fluid, 16
 FeSSIF and, 235
 of gastric fluid
 fasted state, 8
 fed state, 8–9
 of intestinal fluid
 fasted state, 14
 fed state, 14
- Caco-2 cells, 74, 139, 142, 144
- Caco-2 monolayer, 23
 intestinal permeability, 26, 33
- Caco-2 permeability data, 311, 315
- Caco-2 system
 applications of, 181
 description of, 179–180
 FaSSIF and, 194
 mechanistic transport studies in, examples of, 181, 182
 pH and, 193
 setup of, 179–180
 standardization of, 181–182
- Caffeine, 256
- Carbohydrates, 130
 digestion, 10, 15
 hydrolysis of, 251
 malabsorption, 130
 ratio, 228, 250
- Carboxylesterases (CES), 79
- Carboxypeptidase, 113
- CAT model. *See* Compartmental absorption and transit (CAT) model
- CCK. *See* Cholecystokinin (CCK)
- CD. *See* Crohn's disease (CD)
- Cecum, 4
- Celiac disease, 133
- CES. *See* Carboxylesterases (CES)
- Chief cells, in stomach, 7

Children

- brush border, 114–115
- dosage form development in, 120
- drug absorption in, 116
 - age dependance of, 117–119
- drug prescribing in, 109
- extemporaneous products in, 120
- gastric emptying, 109–111
- gastric pH in, 109
- hepatic enzymes, 114–115
- intestinal permeability, 111–112
- oral formulation development for, 116–121
- small intestinal surface area, 112–113
- small intestinal transit, 111
- theophylline formulation in, 121
- transporters, 114–115
- upper GI tract, luminal composition in, 113–114

Chloride

- in gastric fluid, 9
- in intestinal fluid, 14

CHMP. *See* Committee for Medicinal

Products for Human Use (CHMP)

Cholecystokinin (CCK), 49, 92

Chylomicrons, 10, 13, 98

Chyme, 3, 11

Ciprofloxacin, 100

Class I drugs, 208, 209, 212, 213, 219, 220

Class III drugs, 207, 209, 210, 214, 219, 220

Classification of drugs, criteria for, 139–140

- dissolution and, 140
- permeability and, 139–140
 - bioavailability (BA) in, 139
- drug metabolism and, 140
- solubility and, 139. *See also* Intrinsic dissolution rate (IDR)

Claversal[®], 254 $C_{\max}$. *See* Plasma concentration ($C_{\max}$)CMC. *See* Critical micelle concentration (CMC)

Colipase, 95

Colon

- ascending, 4
- bile salts in, 16
- descending, 4
- description of, 15–16
- distribution of materials in, 57
- drug solubility in, 163–165
- fluid composition, 16–18
- gas, 55–57
- motility and transit in, 18
- role, 15–16

[Colon]

- time of dosing, 58–59
- transit times, 4–5
- water absorption by, 1
- water content
 - drug dispersion in, 54
 - motility changes in, 55

Colonic drug absorption

- drug degradation and, 274–275
- gut wall metabolism and, 275
- permeability and, 272–274
- preclinical risk assessment of, 272–275
- solubility and, 274

Committee for Medicinal Products for Human Use (CHMP), 365

Compartmental absorption and transit (CAT) model, 301, 338–339

Computer-based absorption models

- applications in drug research and discovery, 342–351
- overview, 338
- theoretical basis of, 339–342

Concentration-time curve (AUC), 90, 356–357

Contiphyllin[®], 260–262

Critical micelle concentration (CMC), 227

Crohn's disease (CD), 132, 133

mesalazine and, 252–253

Cyclosporine, 70, 115

CYP. *See* Cytochromes P450 (CYP)

CYP3A4, 29, 350

CYP3A enzymes, 70–72

- dietary substances in, 74
- intestinal *vs.* hepatic, 75
- in vivo studies, 70–71
- localization of, 73
- with mRNA, 70
- pathophysiologic conditions for, 74–75
- substrate midazolam, 71
- therapeutic agents in, 74

CYP3A4 proteins, 72–73

CYP3A5 proteins, 72–73

CYP3A4 *vs.* CYP3A5, 72–73

CYP2C9, 76

CYP2C19, 76–77

CYP2D6

- in human intestinal, 77

CYP enzymes. *See* Cytochrome P (CYP) enzymes

CYP4F, 78

CYP2J2, 77–78

Cyprotex model, 339, 341

Cytochrome P450, 29

Cytochrome P (CYP) enzymes, 171

Cytochromes P450 (CYP), 69–70

- DAG. *See* Diacylglycerides (DAG)
- Danazol, 158, 203
- Deoxycholic acid, 114
- Dermatomyositis, 134
- DESI. *See* Drug efficacy study implementation (DESI)
- Design space
- definition of, 392
 - identification of, 392
- Diacylglycerides (DAG), 91
- Diarrhea, 16, 134
- Didanosine, 326
- Dietary residues, 17
- Diffusion chambers
- applications of, 185–186
 - description of, 184–185
 - pH and, 193
 - use of FaSSIF in, 194
- Digestion
- of intestinal lipid, 95
 - in small intestine
 - carbohydrates, 10
 - fats, 10
 - proteins, 10
- in stomach, 91
- fat, 5
 - proteins, 5
- Digoxin, 2, 111
- Dipyridamole
- in fasted state, 158, 160
 - in fed state, 160
- Dispersion, 54
- Dissolution data, 231
- Dissolution media, 248
- class I drugs, 224
 - class II drugs, 224
 - class III drugs, 224
 - class IV drugs, 224
 - fasted state, simulating upper GI tract in, 249–250
 - SGF and, 249
 - SIF and, 249–250
- fed state, simulating upper GI tract in, 250–251
- FeSSIF medium, 250–251
 - milk and complete nutrition products (Ensure[®] Plus), 250
- proximal colon, simulating conditions in, 251
- small intestine, drug dissolution in, 231–235
- FaSSIF and, 233–234
 - FeSSIF and, 234–235
 - snapshot media and, 234
- [Dissolution media]
- stomach, drug dissolution in, 225–231
 - FaSSGF and, 226
 - FeSSGF and, 229
 - snapshot and, 229
 - UHT-milk and, 229–231
- Dissolution rate, 155–156
- of drugs in SIF, 249
 - food intake and, 248
- Dissolution test
- device, 238
 - methods
 - biorelevance of, objectives for improving, 245–246
 - dissolution media and, 248–251
 - official, 245
 - test equipment, 246–248
- Dogs
- RZ-50 in, 229
- “Dose dumping,” 244
- Dose number, 146, 148, 149
- Drugs. *See also* Tablets
- absorption. *See also* Transporters
 - equation for, 140–141
 - factors affecting, 22–23, 24
 - Fick’s first law and, 24
 - food effects on, 147–148. *See also* Noyes-Whitney equation
- intestinal fluid and, 14
- intraluminal solubility and, 155–157
 - luminal concentration and, 24–25, 29
 - permeability coefficient and, 24
 - P-glycoprotein and, 13
 - pH and, 30
 - process of, 21–23
 - rate-limiting step of, 144, 146, 147, 149
 - rate of, 141
 - in small intestine, 13
 - solubility and, 24
 - in stomach, 7
 - structural properties of drug molecules and, 22
- class I drugs, 208, 209, 212, 213, 219, 220
- class III drugs, 207, 209, 210, 214, 219, 220
- delivering
- advantageous cases, 2
 - disadvantageous cases, 2
- development, phases of, 146–147
- formulation, 141, 142, 143, 146
- higher molecular weight and, 27
 - interaction, 142, 147
- labeling and BE, 140
- metabolism, permeability classification and, 140

- [Drugs]
 - oral bioavailability $F(\%)$, 21
 - permeability of, 206, 207, 208, 209, 212, 216, 221
 - rapid dissolution of, 207, 208, 209, 210, 211, 212, 213, 219, 220
 - solubility of, 207, 208, 211, 214
 - testing, 138, 143, 145, 149
 - transport
 - mechanisms, 25–27
 - metabolism during, 29–30
 - process of, 23–25
 - requirements, 25–27
- Drug degradation
 - colonic drug absorption and, 274–275
- Drug efficacy study implementation (DESI), 220
- Drug permeability
 - assessment of, 139–140
 - cell models in, 139–140
 - excipient effect on, 144–145
- Drug release rate, dissolution and, 143–144
- Drug-release testing
 - USP apparatus 3, 246–247
- Drug solubility
 - in colon, 163–165
 - in fasted small intestine, 160–161
 - in fasted stomach, 157–159
 - in fed small intestine, 161–163
 - in fed stomach, 159–160
 - pH and, 139
- “Drug wastage,” 252
- Duodenum, 3, 93
- “Early” phase, gastric digestion process and, 229, 234
- Effective permeability coefficient
 - versus* apparent permeability coefficient, 174–175
- Efflux
 - of digoxin, 350
 - intestinal, 171–172
 - mechanisms. *See* P-glycoproteins
- ELAN nanomilling technology, 313
- EMA. *See* European Medical Evaluation Office (EMA); European Medicines Agency (EMA); European Medicines Evaluation Agency (EMA)
- EMA draft guidelines, 210–211
- ENS. *See* Enteric nervous system (ENS)
- Ensure[®] Plus, 159, 250
 - as intragastric medium, 159
- Enteric coated formulation, 265
- Enteric nervous system (ENS), 51
- Enterocytes, 10, 12–13
- Enterohepatic cycling, 2
- Enzymes
 - amidases/proteases, 29
 - amylase, 10
 - CYP3A4, 29
 - cytochrome P450, 29
 - gastric lipase, 5
 - glucuronidases, 29
 - GST, 29
 - hCE1, 30
 - hCE2, 30
 - in intestinal wall, 68–83
 - carboxylesterases (CES), 79
 - CYP1A1, 75–76
 - CYP3A, 70–72
 - CYP3A4 *vs.* CYP3A5, 72–73
 - CYP2C19, 76–77
 - CYP2D6, 77
 - CYP4F, 78
 - CYP2J2, 77–78
 - cytochromes P450 (CYP), 69–70
 - flavin monooxygenases (FMO), 79
 - in human small intestine, 69
 - N-acetyltransferase (NAT), 81
 - S-transferase (GST), 81
 - sulfotransferases (SULT), 79–80
 - UDP-Glucuronosyl Transferases (UGT), 80–81
 - lipase, 7, 10
 - lipases/esterases, 29
 - pepsin, 5, 7, 9
 - pepsinogen, 7
 - proteases, 10
 - sulfotransferases, 29
 - SULT, 29
 - UGT, 29
- Epoxide hydrolases, 79
- Equilibrium solubility, 155–156
- ER formulation. *See* Extended release (ER) formulation
- Esophageal transit, 42–43
- Esophagus, transit times, 4
- Ethoxyresorufin O-deethylation, 75
- European guidance, 375
 - BCS-based biowaiver
 - current status, 377
 - formulation considerations, 376–377
 - in vitro* dissolution, 376
 - permeability, 376
 - solubility, 376

- European Medicines Evaluation Agency (EMA), 108, 138, 206, 208, 228, 358
 - approach for evaluation of HV drugs, 365
 - guideline
 - metabolites in BE assessment, 367
- Everted intestinal sacs/rings
 - applications of, 183–184
 - description of, 183
 - limitations of, 184
- Extended-release (ER) dosage form
 - asthma and, 258
 - predicting drug release from, 258–262
 - theophylline, 258–262
- Extended release (ER) formulation
 - plasma concentration and time profiles for, 265, 267
- FaSSIF. *See* Fasted state-simulated intestinal fluid (FaSSIF)
- Fasted small intestine
 - drug solubility in, 160–161
- Fasted state
 - bile acids, 14
 - buffer capacity
 - of small intestine, 14
 - of stomach, 8
 - drug dissolution medium in, 226–227
 - gastric fluid osmolality, 9
 - pH
 - of small intestine, 13–14
 - of stomach, 8–9
 - stomach motility, 9
 - surfactants in, 226
- Fasted state-simulated intestinal fluid (FaSSIF), 158, 161, 194, 225, 226–227, 310
- Fasted stomach
 - drug solubility in, 157–159
- Fat digestion
 - in small intestine, 10
 - in stomach, 5
- Fat malabsorption, 128–130
- FDA. *See* Food and Drug Administration (FDA)
- FDA guidance, 211, 218
- Federation Internationale Pharmaceutique (FIP), 382
- Fed small intestine
 - drug solubility in, 161–163
- Fed state
 - bile acids, 14
 - buffer capacity
 - of small intestine, 14
 - of stomach, 8–9
 - [Fed state]
 - drug dissolution medium in, 228–231
 - pH of stomach and, 8–9
 - tablets, disintegration process of, 228
- Fed-state gastric emulsion system (FSGES), 228
- Fed state-simulated intestinal fluid (FeSSIF), 163, 194, 225, 310
- Fed stomach
 - drug solubility in, 159–160
- Felodipine, 74, 158, 266
- FeSSIF. *See* Fed state-simulated intestinal fluid (FeSSIF)
- Fexofenadine, 99
- FFA. *See* Free fatty acid (FFA)
- Fick's first law, 24, 173
- FIM formulation. *See* First-in-man (FIM) formulation
- FIP. *See* Federation Internationale Pharmaceutique (FIP); International Pharmaceutical Federation (FIP)
- First-in-man (FIM) formulation
 - decision tree, 304
 - development flow charts, based on BCS, 305
- First-pass extraction
 - in liver and intestine, prediction of, 350
- Flavin monooxygenases (FMO), 79
- Flow-through apparatus. *See* USP apparatus 4
- Flow-through cell. *See* USP apparatus 4
- Fluid. *See also* Water
 - intake, GI tract and, 1
 - transit times and, 4
 - transport of, MRI and, 238
- FMO. *See* Flavin monooxygenases (FMO)
- Food
 - effect of, 1
 - fat-rich, motility and, 4, 9
 - tablets, nondisintegrating, 10
 - volume, in stomach, 2
- Food and Drug Administration (FDA), 108, 138, 206, 250
- Food effects
 - on drug absorption
 - BCS class 1 drugs, 96–97
 - BCS class 2 drugs, 97–99
 - BCS class 3 drugs, 99
 - BCS class 4 drugs, 99–100
 - and dosage form, 100–101
 - and food composition, 100
 - food intake, physiological effects of, 90–95
 - formulation to eliminate, 101–102
 - overview, 90
 - prediction of, 347–348

- Food intake, physiological effects of, 90–95
 digestion in stomach, 91
 intestinal events, 92–95
- Formulation development. *See also*
 Immediate-release (IR) formulation;
 Modified-release (MR) formulations
 absorption modeling in, 301
 BCS principles in, 297–299
 current state of, 296–297
 future aspects, 329–333
 in vitro dissolution testing in, 300
 key challenges in, 296–297
 preclinical
 animal models in, 300–301
 assisting, 345–346
- Four-compartment system, 161
- Free fatty acid (FFA), 91
- FSGES. *See* Fed-state gastric emulsion system (FSGES)
- f2 test, 138, 143
- Furanocoumarins, 74
- Fybogel, 56
- γ -camera, 41
- γ -scintigraphy, 41, 49, 51, 291
- Gall bladder, 2, 3
- Gastric acid, 91. *See also* Gastric fluid
- Gastric emptying (GE), 2, 43–50, 92. *See also*
 Transit times
 in children, 109–111
 disintegrating dosage forms, 44
 factors for, 4
 homogeneity after food, 45
 impact of food on, 92
 in vitro dissolution rate, 47
 in vivo dissolution rate, 47
 for larger solids, 9–10
 nondisintegrating forms, 43–44
 postprandial phase and, 2
 prolongation of, 110–111
 rate, in fasted state, 158
 scintigraphy, 48
 for small solids, 9
 soft gel formulation, 44
- Gastric fluid. *See also* Hydrochloric acid; Pepsin
 chloride in, 9
 composition of, 8–9
 defense mechanisms of, 5
 in fasted state, 8–9
 in fed state, 8–9
 osmolality of, 9
 parietal cells and, 7
 potassium in, 9
 protein digestion and, 5
- [Gastric fluid]
 sodium in, 9
 surface tension of, 9
 volume of, 2
- Gastric lipase, 5, 91
- Gastrin, 7, 8
- Gastrointestinal disease
 AIDS enteropathy, 133–134
 celiac disease, 133
 Crohn's disease, 133
 malabsorption, 127–130
 aging, 135
 drug- and irradiation-induced, 135
 maldigestion, 130–133
 overview, 127
 small-bowel resection, 135–136
 systemic diseases, 134–135
- Gastrointestinal (GI) tract, 90, 168–169.
See also Large intestine; Small intestine;
 Stomach
 delivering drugs
 advantageous cases, 2
 disadvantageous cases, 2
 dimensions of, 2–4
 function of, 1–2
 immune system of, 2
 mucosa. *See* Gastrointestinal mucosa
 permeability assessment
 based on transport curves, 172–174
 bidirectional transport, 175–176
 biorelevance of model systems for,
 190–192
 concentration-dependent, 176
 effective *versus* apparent coefficient,
 174–175
 experimental models for, 176–189
 nonrelevant barriers for, in model
 systems, 191–192
 single time point, 174
 under nonsink conditions, 174
 transit times, 4–5
- Gastrointestinal mucosa
 as barrier to drug permeation
 biochemical barrier, 171–172
 physical barrier, 170–171
 UWL, 191
 morphological characteristics of, 169
 small intestine, surface area of, 170
- Gastrointestinal transit
 colon, water content, 54–55
 effects of age, 59–60
 effects of gender, 59–60
 esophageal transit, 42–43
 gastric emptying, 43–50
 overview, 41–42

- [Gastrointestinal transit]
 - SITT, 50
 - in small intestine, 50–54
- GastroPlus™ software, 33, 301, 338, 339, 340, 342
 - use of, 343, 345–346
- Gastroretentive systems, 49
- G cells, 8
- Gelatin capsules, 43
- GI. *See* Gastrointestinal (GI) tract
- Glibenclamide tablets, food effects in, 235
- Glucuronidases, 29
- Glutathione S-transferase (GST), 81
- Glutathione transferase (GST), 29
- Goblet cells, 12
- Griseofulvin, 99
- GR253035X, 231
- GST. *See* Glutathione transferase (GST); Glutathione S-transferase (GST)
- Gut. *See* Gastrointestinal (GI) tract
- Gut wall metabolism
 - enzymes, in intestinal wall, 68–83
 - intestinal first-pass metabolism, 67–68
 - overview, 66
 - regional drug absorption and, 275
- HCE1, 30
- HCE2, 30
- Henderson–Hasselbalch model, 340
- Hepatic enzymes, 114–115
- Herbivores, 5
- HHS-FDA. *See* U.S. Department of Health and Human Services, Food and Drug Administration (HHS-FDA)
- High drug permeability, defined, 138
- Highly variable drug (HVD), 212
 - BE assessment and, 358–365
- “High solubility”
 - definition of, 374
- High-solubility drugs, 208, 211, 214
 - defined, 149
- HIV. *See* Human immunodeficiency virus (HIV)
- HIV protease inhibitors, 72
- HMG-CoA reductase inhibitors, 74
- Homeostasis, GI tract in, 1–2
- Hormone replacement therapy (HRT), 60
- Hormones
 - gastrin, 7, 8
- Housekeeper wave, 5
- HPePT1 transporters, 28
- HRT. *See* Hormone replacement therapy (HRT)
- Human immunodeficiency virus (HIV), 134
- Human intestinal fluid
 - characteristics of, 94
- Humans
 - prediction of oral pharmacokinetics in, 346–347
- HVD. *See* Highly variable drug (HVD)
- Hydrochloric acid, 7
 - in SGF medium, fasted state, 249
- Hydrodynamics, 46
 - intraluminal, simulation of, 235–240
 - in in vitro, 236–237, 238
 - in in vivo, 237
 - stress, 47
 - USP apparatus 2 in, 236
 - USP apparatus 3 in, 236, 248
 - USP apparatus 4 in, 236, 248
- Hydrogen bonding potential, drug transport and, 25–26
- 1'-Hydroxymidazolam, 71
- Hypothyroidism, 134
- IACUC. *See* Institutional Animal Care and Use Committee (IACUC)
- IBAT transporters, 28
- IBD. *See* Inflammatory bowel diseases (IBD)
- ICH. *See* International Conference on Harmonization (ICH)
- IDEA software, 342
- IDR. *See* Intrinsic dissolution rate (IDR)
- Ileal brake, 50
- Ileocecal valve
 - fluid transport and, 238
 - solid transport and, 238
- Ileum
 - bile salts reabsorption and, 12
 - fluid absorption in, 1
 - role, 3–4
- IMMC phase. *See* Interdigestive migrating myoelectric complex (IMMC) phase
- Immediate-release (IR) formulation, 206, 210, 211, 212, 213, 214, 217, 218, 219, 220
 - animal models, 318
 - challenges and limitations, 327–329
 - common models, 320–321
 - dog model, 325–327
 - importance of characterization, 319
 - oral clinical formulations, 321–325
- class I drugs, 224
- class III drugs, 224
- development of
 - common strategies for, 303–307
 - typical work flow in, 302–303

- [Immediate-release (IR) formulation]
 - formulation, 301
 - in vitro screening of
 - challenges and limitations, 316–318
 - clinical formulations, 311–316
 - common methods, 309–311
 - in vitro characterization, importance of, 307–309
 - plasma concentration and time profiles for, 265, 267
- In-111. *See* Indium-111 (In-111)
- IND. *See* Investigational New Drug (IND)
- Indium-111 (In-111), 43
- Individual BE, 359–360
- Inflammatory bowel disease (IBD), 252
- Inhibitors
 - use of, in bidirectional transport, 176
- In situ intestinal perfusion model
 - applications of, 187–188
 - description of, 186–187
 - drawback of, 188
- Institutional Animal Care and Use Committee (IACUC), 218
- Institutional Review Board (IRB), 219
- INTELLIPHARM[®] PKCR, 301
- Intellipharm software, 339, 341
- Interdigestive migrating myoelectric complex (IMMC) phase
 - in small intestine, 13
 - in stomach, 9
- International Conference on Harmonization (ICH)
 - QbD in
 - Q10 (pharmaceutical quality system), 393
 - Q8 (product development), 392–393
 - Q9 (quality risk management), 393
- International Pharmaceutical Federation (FIP), 207
- International Pharmacopoeia, The*, 379
- Intestinal first-pass metabolism
 - clinical implications of, 67–68
- Intestinal fluids, 13–15
- Intestinal membranes. *See also* Large intestine; Small intestine
 - active transport, 27–29
 - metabolism during transport, 29–30
 - processes of drug transport, 23–25
- Intestinal perfusion
 - in situ model, 186–188
- Intestinal permeability, 26, 33
 - caco-2 monolayer, 26, 33
 - in children, 111–112
 - of drugs, 146, 150
- Intestinal pH, 13
 - in fasted state
 - drug dissolution, 233
 - in fed state
 - drug dissolution, 235
- Intestine
 - prediction of first-pass extraction in, 350
- Intragastric solubility
 - in fasted state
 - danazol and, 158
 - dipyridamole and, 158
 - felodipine and, 158
 - miconazole and, 158
 - in fed state
 - dipyridamole and, 160
 - Ensure plus[®], 159
 - ketoconazole and, 160
 - “snapshot” media and, 159
- Intralipid, 52
- Intraluminal drug release prediction, with apparatus. *See* Apparatus
- Intraluminal solubility
 - drug absorption and, 155–157
- Intrinsic dissolution rate (IDR), 139
- Intubation techniques, 276
- Investigational New Drug (IND), 208, 374
- In vitro characterization
 - during formulation development, 307–309
- In vitro dissolution testing, 141, 145, 149, 150, 349
 - in formulation screening, 300
 - for MR formulations
 - factors influencing, 278
 - preclinical models, 286–287
 - in prototype selection and optimization, 277–284
 - refining biorelevant dissolution methods, 284–285
 - robustness *vs.* physiological factors, 285–286
- In vitro-in vivo correlation (IVIVC), 224, 300
 - biowaiving based on, 388–391
 - case examples: ER formulations, 259–262
 - case examples: novel pH- and time-based multiunit colonic delivery system, 256–258
 - dissolution and, 256–258
 - FDA guidance on, 390–391
 - FeSSGF and, 229
 - four levels of, 390–391
 - preliminary
 - for MR formulation, 270–271
 - role of, 392

- In vitro studies, 212–221
 advantages of
 cost reduction, 213–215
 direct assessment, 216–217
 ethical considerations and, 218–219
- In vivo imaging
 for MR formulations, 291–292
- In vivo methods, for MR formulation
 bioavailability studies
 design, 288–290
 evaluation, 290–291
 in vivo imaging, 291–292
- In vivo studies, 212–221
- IR. *See* Immediate-release (IR);
 Immediate-release (IR)
- IRB. *See* Institutional Review Board (IRB)
- IR formulation. *See* Immediate-release (IR)
 formulation
- Itraconazole, supersaturation of, 156
- IVIVC. *See* In vitro-in vivo correlation (IVIVC)
- Jejunum
 bile salts reabsorption and, 12
 fluids absorption in, 1
 in infants, 112
 role, 3
- Kaletra[®], 102
- Kanamycin, 135
- Ketoconazole, 275
 in fed state, 160
- Lactobacillus plantarum*, 56
- Lactulose, 111
- Large intestine, 4
 “Late” phase, gastric digestion process and,
 229, 234
- LDE. *See* Lyophilized dry emulsion (LDE)
- Leucotriene receptor (LTD4), 58
- Levy plot, 258
- LFC. *See* Liquid-filled capsule (LFC)
- Ligament of Treitz, 3
- Lipase
 in small intestine, 10
 in stomach, 7
- Lipases/esterases, 29
- Lipinski’s “rule of five,” 26
- Lipophilicity, drug transport and, 25
- Liquid-filled capsule (LFC), 299, 300, 308, 314
- Lithocholic acid, 114
- Liver
 prediction of first-pass extraction in, 350
- Log *D* model, 340
- L-rhamnose, 111
- LTD4. *See* Leucotriene receptor (LTD4)
- Luminal concentration, drug absorption and,
 24–25, 29
- Luminal enzymes, 113
- Lyophilized dry emulsion (LDE), 102
- MAD concept. *See* Maximum absorbable dose
 (MAD) concept
- Madin-Darby canine kidney (MDCK), 31, 139,
 182–183
- MAD studies. *See* Multiple ascending dose
 (MAD) studies
- MAG. *See* Monoacylglycerol (MAG)
- Magnetic marker monitoring, 291
- Magnetic resonance imaging (MRI), 237–238,
 291
- Malabsorption, 127–130
 aging, 135
 carbohydrate, 130
 defined, 128
 diseases resulting in, 131–132
 drug- and irradiation-induced, 135
 fat, 128–130
 pancreatic insufficiency, 134
 pathophysiology of, 129
 protein, 128
 in small-intestinal resection, 136
 syndromes, 128
- Malabsorption-maldigestion syndrome, 131
- Maldigestion, 130–133
 diseases resulting in, 131–132
 pathophysiology of, 129
- Mannitol, 111
- Manufacturing scale-up
 MR formulation, 271–272
- Mapping, concept of, 391
- Matrix tablets, 244
- Maximum absorbable dose (MAD) concept,
 344
 calculation, 299
- MCT transporters, 28
- MCT1 transporters, 28
- MDCK. *See* Madin-Darby canine kidney
 (MDCK)
- MDR1 transporters, 27–28
- Meal. *See* Fluid; Food
- Medical Products Agency (MPA), 142
- Mesalazine, 252–256
- Metabolism
 in drug absorption, 21–22, 29–30
- Metformin absorption, 50
- Methane, 57

- Metoprolol, 71, 276
- Micelle formation, 129
- Michaelis-Menten equation, 24
- Michaelis-Menten model, 350–351
- Miconazole, 158
- Microvilli, in absorption, 7, 10, 12
- Midazolam, 71, 97, 115
- “Middle” phase, gastric digestion process
and, 229, 234
- Migrating motor complex (MMC), 49, 91
- Milk
as dissolution medium, 250
as intragastric medium, 159–160
UHT, 228, 229–231
undigested, 231
undigested, intragastric medium, 160
- Mixed model, 362–363
- MK-0869 (aprepitant), 326–327
- MMC. *See* Migrating motor complex (MMC)
- Modified-release (MR) formulations, 238
basic technologies for, 267–268
development of, 267, 269–270
drug solubility and, 279–280
efficacy and tolerability, improvement of,
266–267
in vitro screening, 276
preclinical models, 286–287
prototype selection and optimization, in
vitro dissolution testing in, 277–284
refining biorelevant dissolution methods,
284–285
robustness *vs.* physiological factors,
285–286
in vivo methods
bioavailability studies, design and
evaluation aspects of, 288–291
in vivo imaging, 291–292
osmolality/ionic strength and, 282
overview, 265–267
screening and evaluation
development and target pharmaceutical
profile, 269–270
manufacturing scale-up, 271–272
prototype optimization and preliminary
IVIVC, 270–271
prototype selection, 270
USP apparatus 3 in, 246–247
USP apparatus 4 in, 247–248
- Monoacylglyceride, 95
- Monoacylglycerol (MAG), 93
- “Monographed dissolution tests,” 244
- Motility
adequate fluid and, 4
caloric load and, 4, 9
in colon, 18
- [Motility]
fasted state and, 15
position and, 4
in small intestine, 15
in stomach, 9–10
IMMC phases, 9
presence of food, 9
for supine patients, 4
tablets, nondisintegrating, 4
- MPA. *See* Medical Products Agency (MPA)
- MR formulations. *See* Modified-release (MR)
formulations
- MRI. *See* Magnetic resonance imaging (MRI)
- MRP2 transporters, 27–28
- Multiple ascending dose (MAD) studies, 270,
277, 299, 344
- Mylan Pharmaceuticals, 141
- N-acetyltransferase (NAT), 81
- NAT. *See* N-acetyltransferase (NAT)
- NCE. *See* New chemical entity (NCE)
- NDA. *See* New Drug Application (NDA)
- Neomycin, 135
- Neoral, 102, 314
- New chemical entity (NCE), 139, 265
- New Drug Application (NDA), 147, 209, 374
- Nitrofurantoin, 49
- Nonpolar surface area (NPSA)
drug transport and, 25–26
- Noyes-Whitney equation, 47, 147, 339, 341
- Noyes-Whitney theory, 155
- NPSA. *See* Nonpolar surface area (NPSA)
- Nutrients absorption, 2, 3–4, 10
- OATP, transporters, 28
- OCT, transporters, 28
- OCTN2, transporters, 28
- Official dissolution test methods, 245
- Oral bioavailability, 67
of drug, 21
selected drugs with low and variable, 67
- Oral drug absorption
predictions and simulations of, 31–34
process of, 21–23
- Oral drug therapy, 68
- Oropharynx, 43
- Osmolality, 93
colonic fluid, 17
FeSSIF and, 235
gastric fluid, 9
intestinal fluid, 14
- Osmotic diarrhea, 132
- Oxybutynin, 275

- Paddle assembly. *See* USP apparatus 2
- P-aminosalicylic acid (PAS), 135
- PAMPA. *See* Parallel artificial membrane permeability assay (PAMPA)
- Pan American Health Organization Working Group on Bioequivalence, 381
- Pancreas, 2, 3. *See also* Pancreatic juice
- Pancreatic enzymes, 113
- Pancreatic juice
- amylase, 10
 - lipases, 10
 - proteases, 10
- Pancreatin, 47
- Parallel artificial membrane permeability assay (PAMPA), 178, 340
- applications of, 179
 - use of pH gradient in, 193
 - UWL and, 191
- Parietal cells, 7
- PAS. *See* P-aminosalicylic acid (PAS)
- PAT1 transporters, 28
- PBPK. *See* Physiologically based pharmacokinetic (PBPK)
- PD. *See* Potential difference (PD)
- PEG. *See* Polyethylene glycol (PEG)
- Pellets, 244
- Penicillin G, 109
- Pentasa[®], 256
- Pepsin, 113
- concentrations, 9
 - drug dissolution in fasted state, 226
 - drug dissolution in fed state, 230–231
 - in SGF medium, fasted state and, 249
 - in stomach, 5
 - surface tension and, 226
- Pepsinogen, 7, 91
- Peptide YY (PYY), 51
- PePT1 transporters, 28
- Peristalsis, 235–236
- Permeability
- colonic drug absorption and, 272–274
 - in drug absorption, 23–24, 33
 - measurements, 33
 - pH and, 30
- Permeability assessment
- biorelevance of media used during, 192
 - fasted-state simulation, 194
 - fed-state simulation, 194
 - pH, 193
 - schematic representation of, 193
 - sink conditions, 195–196
 - experimental models for
 - biorelevance of, 190–192
 - Caco-2. *See* Caco-2 system
 - diffusion chambers, 184–186
 - [Permeability assessment
 - experimental models for]
 - everted intestinal sacs/rings, 183–184
 - in situ intestinal perfusion model, 186–188
 - marker compounds and, 188–189
 - MDCK cells, 182–183
 - nonrelevant barriers for, 191–192
 - PAMPA, 178–179
 - strengths and limitations of, 176–177
 - of GI tract
 - based on transport curves, 172–174
 - bidirectional transport, 175–176
 - concentration-dependent, 176
 - effective *versus* apparent coefficient, 174–175
 - under nonsink conditions, 174
 - single time point, 174
 - silico method of, 139, 150
- Peroxisome proliferator-activated receptor (PPAR), 218
- Perspex capsule, 51
- PET. *See* Positron emission tomography (PET)
- Peyer's patches, 2, 4, 14
- PF. *See* Polarity factor (PF)
- pH
- of colon, 16
- drug absorption and, 30
- in fasted state
- SGF medium, 249
- SIF medium, 249
- in fed state
- FeSSIF medium, 251
- milk dissolution medium, 250
- food-induced, 98
- in proximal colon, 251
- of test medium, drug release from the tablet formulations and, 253
- Pharmacokinetics, 142, 206, 207, 211, 214, 215, 216, 217, 220
- Phenacetin, 76
- pH of stomach, 5
- after liquid meal, 8
- after solid meal, 8
- contents of meal and, 8
- in fasted state, 8–9
- drug dissolution, 226
- in fed state, 8–9
- drug dissolution, 231
- Phospholipids (PL), 93
- Photoshop, 56
- pH shift method, 279

- Physiologically based pharmacokinetic (PBPK)-based models, 31, 33, 340–341
- Physiologic cholestasis, 114
- PK-Sim[®] (Bayer Technology Services), 301, 339, 341
- PL. *See* Phospholipids (PL)
- Plasma concentration ($C_{\max}$), 356–357
- Polarity factor (PF)
calculation of, 175
interpretation of, 176
- Polar surface area (PSA)
drug transport and, 25–26
- Polyethylene glycol 2000, 53
- Polyethylene glycol (PEG), 33
- Positron emission tomography (PET), 289
- Postoperative syndromes, 132
- Postprandial phase, 2
- Postprandial state, 228–231
- Potassium
in gastric fluid, 9
in intestinal fluid, 14
- Potential difference (PD), 185
- PPAR. *See* Peroxisome proliferator-activated receptor (PPAR)
- Pregabalin, biowaiver for, 141
- Pretprandial state, 227–228
- Probutol, 99
- Prodrugs, 146
- Proteases, 10
- Proteins. *See also* Enzymes; Hormones
BCRP, 27–28
chylomicrons, 13
digestion
in small intestine, 10
in stomach, 5
hPePT1, 28
IBAT, 28
malabsorption, 130
MCT, 28
MCT1, 28
MDR1, 27–28
MRP2, 27–28
PAT1, 28
P-glycoproteins, 2, 13
SLC1, 28
SLC6, 28
SLC7, 28
SLC16, 28
SLC15A1, 28
- Prototype optimization
for MR formulation, 270–271
in vitro dissolution testing in, 277–284
- Prototype selection
for MR formulation, 270
in vitro dissolution testing in, 277–284
- PSA. *See* Polar surface area (PSA)
- Pulsincap, 55, 57
studies for dosing, 58
- Pylori-duodenal junction, 43
- PYY. *See* Peptide YY (PYY)
- Q8, product development, 392–393
- Q9, quality risk management, 393
- Q10, pharmaceutical quality system, 393
- QbD. *See* Quality by Design (QbD)
- QbR. *See* Quality-based Review (QbR);
Question-based Review (QbR)
- “QGut” model, 341
- Quality-based Review (QbR), 149
- Quality by Design (QbD), 149, 212, 240, 391–392
in ICH guidances
Q10 (pharmaceutical quality system), 393
Q8 (product development), 392–393
Q9 (quality risk management), 393
- Question-based Review (QbR), 212
- Rapid dissolution of drug, 140, 207, 209, 210, 211, 212, 213, 219, 220
defined, 208
- Rat
transporters in, 28
- RC tablets. *See* Roller-compacted (RC) tablets
- Reciprocating cylinder. *See* USP apparatus 3
- Reference listed drug (RLD), 218
- Remote control capsules, 276
- Response surface, 391
- Reynolds number, 236
- Riboflavin, 49
- RLD. *See* Reference listed drug (RLD)
- Roche compound (RZ-50), 229
- Roller-compacted (RC) tablets, 308
- Rotatable bonds, drug transport and, 25–26
- Rugae, 5
- Rule of thumb, 26
- R-warfarin, 76
- SAD. *See* Single ascending dose (SAD) studies
- Saliva, peristalsis and, 235
- Salofalk[®], 254
- Sandimmune[®], 314
- Sandwich-based meal, 45
- SAPP. *See* Sodium acid pyrophosphate (SAPP)
- SAR. *See* Structure-activity relationship (SAR)
- Scaled average bioequivalence (ABEsc), 362

- Scale-Up and Post-Approval Changes—
 immediate release (SUPAC-IR), 138
- Scale-Up and Post-Approval Changes
 (SUPAC) guidance, 373, 390
- SCC. *See* Short circuit current (SCC)
- SCFA. *See* Short-chain fatty acids (SCFA)
- Scintigraphy
 gastric emptying, 48
- Scleroderma, 134
- SCoF. *See* Simulated colonic fluid (SCoF)
- SDS. *See* Sodium dodecyl sulfate (SDS)
- Self-microemulsifying drug delivery systems
 (SMEDDS), 102
- SGF. *See* Simulated gastric fluid (SGF)
- Short-bowel syndrome
 defined, 135
- Short-chain fatty acids (SCFA), 15, 55, 251
- Short circuit current (SCC), 185
- Side effects, 244, 252, 253, 256
- SIF. *See* Simulated intestinal fluid (SIF)
- SIFsp. *See* Sine pepsin (SIFsp)
- SimCYP model, 339, 341, 351
- SimCYP software, 33
- Simulated colonic fluid (SCoF), 251
- Simulated gastric fluid (SGF), 226, 310
 in fasted state
 hydrochloric acid in, 249
 pepsin in, 249
 sodium chloride in, 249
 water in, 249
- Simulated intestinal fluid (SIF), 233
 in fasted state, 249–250
- Simulation
 of efflux and uptake transporters in gut,
 350–351
 in rat in early discovery, 342–344
- Simulations Plus, 338, 339
- Sine pepsin (SIFsp), 253
- Single ascending dose (SAD) studies, 270
- Site-specific delivery systems
 to colon, 256–258
 predicting drug release from, 252–252
- SITT. *See* Small intestinal transit times
 (SITT)
- SLC21, transporters, 28
- SLC22, transporters, 28
- SLC15A1 transporters, 28
- SLC1 transporters, 28
- SLC6 transporters, 28
- SLC7 transporters, 28
- SLC16 transporters, 28
- SLS. *See* Sodium lauryl sulfate (SLS)
- Small-bowel resection, 135–136
- Small intestinal surface area, 112–113
- Small intestinal transit in children, 111
- Small intestinal transit times (SITT), 50, 111
 age dependence of, 112
- Small intestine
 absorption of nutrients in, 10
 absorptive mechanisms in. *See* Villi
 in adults, 3
 bacteria in, 1
 description of, 10–12
 dissolution media, dosage form
 performance, 231–235
 duodenum, 3
 ileum, 3
 intestinal fluid, 13–15
 jejunum, 3
 motility, 4, 15, 50–54
 role, 10–12
 stirring in, 50–54
 transit times, 15
 healthy adults and, 4
- SmartPill GI monitoring capsule, 238
- SMEDDS. *See* Self-microemulsifying drug
 delivery systems (SMEDDS)
- “Snapshot” media, 229, 234–235
- Sodium
 in gastric fluid, 9
 in intestinal fluid, 14
- Sodium acid pyrophosphate (SAPP), 144
- Sodium chloride
 in SGF medium, fasted state and, 249
- Sodium dodecyl sulfate (SDS), 282
- Sodium lauryl sulfate (SLS), 226
- Solid oral dosage, 206, 212, 218, 219, 220
- Solid(s)
 equilibrium solubility and, 155
 transport of, 238
- Solubility
 colonic drug absorption and, 274
 MR formulation and, 279–280
- SOLVO™, 28
- Sotalol hydrochloride, biowaiver for,
 141–142
- SPR. *See* Structure property relationships
 (SPR)
- Squamous/columnar epithelial cells, 5–7
- Steatorrhea
 defined, 129
- Stella, 338
- Stomach
 cells
 chief, 7
 G cell, 8
 parietal, 7
 squamous/columnar epithelial, 5–7
 types and function, 5–8
 chyme and, 3, 10, 11

- [Stomach]
 - dissolution media, dosage form
 - performance, 225–231
 - drug absorption in, 7
 - gastric fluid, composition of, 8–9
 - IMMC phases in, 9
 - meal emptying, factor for, 5
 - motility in, 9–10
 - pepsin, 5
 - pH. *See* pH of stomach
 - role, 5
 - transit, 9–10
 - volume of meal in, 2, 5
- Stool, 1, 4, 12, 15, 16, 17
- Structure-activity relationship (SAR), 23, 27
- Structure property relationships (SPR), 23
- Sulfotransferases (SULT), 29, 79–80
 - in human GI tract, 79–80
- SULT. *See* Sulfotransferases (SULT)
- SUPAC guidance. *See* Scale-Up and Post-Approval Changes (SUPAC) guidance
- SUPAC-IR. *See* Scale-Up and Post-Approval Changes—immediate release (SUPAC-IR)
- Supersaturation, 156–157
- Surface tension
 - bile salts and, 226
 - CMC and, 227
 - colonic fluid, 17
 - drug dissolution medium and, 226
 - fasted state gastric fluid, 9
 - in fasted state of SGF medium, 249
 - fasting and fed state intestinal fluid, 14
 - fed state gastric fluid, 9
 - lecithin and, 226
 - pepsin and, 226
 - pure water, 9
 - surfactants and, 226
 - synthetic, 226
- Surfactants
 - drug dissolution in fasted state, 226
 - SLS, 226
 - surface tension and, 226
 - synthetic, 226
 - Triton-X[®] 100, 226
- Systemic diseases, small-intestinal
 - involvement in, 134–135
- Tablets
 - fed state, disintegration process in, 228
 - glibenclamide, food effects in, 235
 - matrix, 244
 - nondisintegrating
 - heavy food and, 10
- [Tablets
 - nondisintegrating]
 - motility and, 4
 - pellets, 244
 - TAG. *See* Triacylglyceride (TAG)
 - Target pharmaceutical profile
 - MR formulation development and, 269–270
 - Tc-99m. *See* Technetium-99m (Tc-99m)
 - Technetium-99m (Tc-99m), 43
 - TEER. *See* Transepithelial electrical resistance (TEER)
 - Testosterone 6 β -hydroxylation, 72
 - Theophylline, 76
 - ER formulations and, 258
 - food effects on, 259–262
 - formulation, in children, 121
 - TIPS. *See* Transjugular intrahepatic portosystemic shunts (TIPS)
 - Torpac[®] minicapsule kit, 320
 - Transepithelial electrical resistance (TEER), 180
 - MDCK cells and, 182
 - Transit times
 - colonic, 18
 - of drug molecules, 23
 - in small intestine, 15
 - in stomach, 9–10
 - Transjugular intrahepatic portosystemic shunts (TIPS), 75
 - Transport
 - across intestinal membranes, 27–29
 - metabolism, 29–30
 - processes of, 23–25
 - mechanisms and their requirements of drug, 25–27
 - Transport curves
 - permeability calculations based on, 172–174
 - Transporters
 - BCRP, 27–28
 - in drug absorption, 23, 25
 - hPePT1, 28
 - IBAT, 28
 - maturation of, 114–115
 - MCT, 28
 - MCT1, 28
 - MDR1, 27–28
 - MRP2, 27–28
 - OAT, 28
 - OATP, 28
 - OCT, 28
 - OCTN2, 28
 - PAT1, 28
 - PePT1, 28
 - in rat intestine, 28
 - SLC1, 28
 - SLC6, 28

- [Transporters]
SLC7, 28
SLC16, 28
SLC21, 28
SLC15A1, 28
Triacylglyceride (TAG), 91
Triglycerides, 129
Triton-X[®] 100, 226
Troglitazone, 231
Tromphyllin[®], 260–262
- UC. *See* Ulcerative colitis (UC)
UDP-Glucuronosyl Transferases (UGT), 80–81
glucuronidation, 80
UGT. *See* UDP-Glucuronosyl Transferases (UGT); Uridin diphosphate glucuronosyltransferase (UGT)
UHT. *See* Ultra-heat treatment (UHT)
Ulcerative colitis (UC)
mesalazine and, 252–253
Ultra-heat treatment (UHT)-milk, 228, 229–231
Unstirred water layer (UWL), 191
Uridin diphosphate glucuronosyltransferase (UGT), 29
U.S. Department of Health and Human Services, Food and Drug Administration (HHS-FDA), 228
U.S. FDA guidance. *See* U.S. Food and Drug Administration (FDA) guidance
U.S. Food and Drug Administration (FDA) guidance, 297, 356, 358
BCS-based biowaiver, 372–373, 386
current status, 375
formulation considerations, 375
in vitro dissolution, 374–375
permeability, 374
solubility, 374
on IVIVC, 390–391
metabolites in BE assessment, 367
narrow therapeutic range drugs according to, 373
U.S. pharmacopeia (USP), 224
apparatus, 138
apparatus 1, 224
apparatus 2, Reynolds numbers and, 236
apparatus 3, 236, 246–247, 248
instrumental parameters for, 252
apparatus 4, 247–248
Reynolds numbers and, 236
USP. *See* U.S. pharmacopeia (USP)
Ussing chamber model, 273
UWL. *See* Unstirred water layer (UWL)
Verapamil, 2, 34, 266
Villi
absorptive cells, 12
absorptive mechanisms and, 7, 10, 12–13
endocrine cells, 12
exocrine cells, 12
goblet cells, 12
Vitamin B₁₂
ileum and, 4
parietal cells and, 7
Vitamin D, 114
Vitamin E, 114
- Wagner-Nelson method, 256
Water
in danazol study, 158
in felodipine study, 158
gastric fluid osmolality and, 9
homeostasis and, 1–2
pepsin concentration and, 9
reabsorption, in colon, 15, 16, 17–18
reuptake, in small intestine, 11–12
in SGF medium, 226
fasted state and, 249
from stomach, in fasted state, 157
stool, 1, 17
surface tension of, 9
Weakly basic drugs, 156
Weibull distribution, 229
Wet-granulated (WG) tablets, 308
WG tablets. *See* Wet-granulated (WG) tablets
WHO. *See* World Health Organization (WHO)
WHO Essential Drug List, 149
WHO guidance. *See* World Health Organization (WHO) guidance
WHO Model List of Essential Medicines, 378
World Health Organization (WHO), 108, 207
activities of, 209–210
World Health Organization (WHO) guidance
BCS-based biowaiver, 377, 386–387
current status, 380
formulation considerations, 380
in vitro dissolution, 379–380
permeability, 379
solubility, 378–379
Xenobiotics, 68
Zidovudine, 115

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ISBN 978-142007733-9



9 781420 077339